Research Article

In vitro anti-hyperglycemic potential of selected medicinal plants and Lactic acid bacterial strains by various biochemical tests

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Abstract | Diabetes mellitus is an endocrinological and metabolic disorder in which production or action of insulin is inhibited. Various pharmacological and non-pharmacological strategies have been adopted to treat the diabetes. Herbal treatment is one of important strategy against type II diabetes. On the other hand probiotic are beneficial fauna of intestine which may exert anti-diabetic effect. The current study was designed to investigate the anti-diabetic potential of medicinal plants (*Adhota vesica*, *Calendula officinalis*, *Cordia latifolia*, *Myristica fragrans*, *Saraca indica*, *Trapa bispinosa*, *Terminalia arjuna* and *Ziziphus jujuba*) by using α-amylase inhibitory assay, non-enzymatic hemoglobin glycosylation, uptake of glucose by the yeast cells, glucose binding affinity. Some probiotic strains of lactic acid bacteria (LAB) were also investigated by glucose adsorption capacity. Methanolic extract of plant at various concentrations and probiotic strains were used in the experiment. Almost all plant extracts showed significant action against respective biochemical test. Among all plants *S. indica* (Leaves) showed effective response among most of the biochemical tests. A probiotic strain *Enterococcus sp.* had highest inhibitory effect (26.2%) on adsorption capacity of glucose at a concentration of CFU 10^{11}

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1. Introduction

Diabetes mellitus is a metabolic disorder in which body ceases the production or action of insulin Jarald *et al*. (2008) regarded as hyperglycemia, glysuria and hyperlipidemia (Porter and Barrett, 2005). Type II *diabetes mellitus* is associated with non-responsiveness of insulin and nearby 50 % destruction of β cells is also observed in autopsy (Donaghue *et al*., 2009).

Global prevalence of *diabetes mellitus* and pre diabetes is 11.1% and 16.0% respectively (Akhtar *et al*., 2016). According to International Diabetes Federation (IDF), in Pakistan there is an alarming situation with 12.8 million diabetic patients and the country will be ranked 8th among the world's top 10 countries with diabetic patients by the year 2035, if the situation remains same (Sherin, 2015). Individuals with diabetes have a threefold increased risk

of developing microvascular and macrovascular complications including retinopathy, renopathy, coronary heart diseases and amputation (Regmi *et al*., 2016).

Globally a major concern about diabetes is its treatment Jarald *et al*. (2008) by pharmacological or non-pharmacological ways (Inzucchi, 2002). In pharmacological aspect the principal therapeutic agents are acarbose, metformin, sulfonylureas and troglitazone (Osadebe *et al*., 2014) although all therapeutic agents have many side effects (DeFronzo, 2004). Majority of world's population (80%) is dependent on herbal treatment so main focus is to search for cheaper, safer and effective natural drugs (Farnsworth *et al*., 1985). According to ethno-pharmacological reports, 1200 plant species have been used by diabetics worldwide (Jarald *et al*., 2008; Piero *et al*., 2012). However, the literature survey presented only 100 plants having hypoglycemic activity (Kar *et al*., 2003).

Present work is based on regionally medicinally important plants including *Adhota veshica* (leaves), *Calendula officinalis* (flowers), *Cordia latifolia*, *Myristica fragrans* (mays), *Saraca indica* (bark and leaves), *Trapa bispinosa* (leaves and seeds), *Terminalia arjuna* (bark and leaves) *ziziphus jujube* (leaves) and selected strains of Lactic Acid Bacteria (LAB) which have demonstrated anti-hyperglycemic potential by various *in vitro* biochemical tests.

According to reported literature, *Adhota veshica*, have been used by traditional Indian and Unani medicinal system (Claeson *et al*., 2000) against diseases of auditory, respiratory and digestive systems (Gangwar and Ghosh, 2014). Traditionally leaves and roots of *Adhota veshica* are reported for the treatment of diabetes (Lone *et al*., 2013). Methanolic extract of *Adhota veshica* (leaves) presented inhibitory effect of α-glucosidase and α- amylase (Gao *et al*., 2008; Agawane *et al*., 2015). *Calendula officinalis* has been commonly used in Indian and Unani medicinal system against diabetes, gastrointestinal, gynecological, and inflammatory diseases (Arora and Majee, 2011).

In another study, ground paste of *Saraca indica* flower formed in milk/honey or decoction of bark is used against diabetes (Mishra *et al*., 2013). *Terminalia arjuna* has not been reported as a hypoglycemic agent but is used as an antimicrobial agent (Ramya, 2008). *Ziziphus jujuba* is a fruit forming species belonging to family Rhamnaceae (Gao *et al*., 2013). Ethanolic leaf extract of *Ziziphus jujuba* is used against diabetes in experimental rats (Shirdel *et al*., 2009). Among probiotics several bacterial strains have also been reported as hypoglycemic agents (Ruan *et al*., 2015). One such study reported hypoglycemic activity of *Lactobacillus* GG cells in streptozotocin-induced diabetic rats (Tabuchi *et al*., 2003). Present study was designed to investigate the anti-diabetic potential of these medicinal plants by various *in vitro* biochemical tests in their comparison.

2. Materials and Methods

2.1. Selection of plants

Selected parts of regionally medicinal important plants are listed in table 1 were collected from different areas of Lahore region.

2.2. Preparation of plants extracts

Selected plants and their parts were washed under tap water and dried under shade. The dried material was crushed into fine powder by grinding machine. The grinded material was soaked in methanol for 24 hours then sonicated with sonicator (Transsonic 470/H, Elma, Singen, Germany). All the extracts were filtered, concentrated with rotary evaporator under reduced pressure and controlled temperature, then stored at room temperature.

Table 1: list of plants used in herbal medicine for the treatment of diabetes

Serial No.	Plant Name	Family Name	Common Name	Part of Plant Used	Traditional Use
1.	*Adhatoda vasica* (L.)	Acanthaceae	Nees	Leaves	Cough, chronic bronchitis, asthma, rheumatism, insecticidal
2.	*Callendula officinalis*	Asteraceae	Gul-e-ashrafi	Flowers	-
3.	*Cordia latifolia* (Roxb.)	Boraginaceae	Sapistan, Lasori	Fruits	Liver tonic, jaundice, dyspepsia, cough, diuretic, astringent, skin diseases
4.	*Mystrica fragrance*	Myristicaceae	Jalvatri	Mace	-
5.	*Saraca indica*	Fabaceae	Ashoka	Leaves, Bark	-
6.	*Trapa bispinosa*	Trapaceae	Singhara	Leaves, Fruits	-
7.	*Terminalia arjuna*	Combretaceae	Arjun	Bark	-
8.	*Ziziphus jujuba* (mill.)	Rhamnaceae	Red date	Fruit	Cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities

2.3. In vitro anti-hyperglycemic potential of methanolic extracts

a) Inhibitory activity of plant extracts on haemoglobin glycosylation

Non-enzymatic glycosylation of haemoglobin was assayed by following previously described protocols (Jovanovic and Peterson, 1981; Chaudhari *et al*., 2013) with slight modifications. Glucose (2%), Gentamycin (0.2%) and Haemoglobin (0.06%) were prepared in 0.01 M phosphate buffer solution whose pH was maintained at 7.4. Freshly prepared above mentioned solutions were mixed in equal ratio (1 ml each). One ml of different concentrations of plant extracts prepared in Dimethyl Sulfoxide (DMSO) were also mixed. All the test tubes were incubated for 72 hours in darkness. The glycosylated haemoglobin in the solution was estimated at 520 nm by UV spectrophotometer. Percentage of inhibition was estimated by following formula;

$$\text{Percentage (\%) inhibition of glycosylated haemoglobin} = \frac{A_s - A_c}{A_s} \times 100$$

Where,
As = Absorbance of sample and
Ac = Absorbance of control

b) Glucose uptake activity by eukaryotic cell (Yeast model)

Instant baker's yeast was purchased from market. Cells were freshly prepared acoording to published protocol (Cirillo,

1962) with some modifications. Yeast cells (10% v/v) suspension was prepared in distilled water. Plant extract or standard drug (1ml) with increasing concentrations of 100 to 500 mg/ml were added to 20 mM glucose solution (1 ml) and incubated at 25°C for 10 minutes. Yeast cells (100 µl) were mixed in the solution by vortex and again incubated at 37°C for 60 minutes. Solutions were centrifuged at 25,000 ×g for 5 minutes after incubation and supernatants were used to measure the glucose in the solution by calorimetric analysis at 500 nm. Acarbose was taken as a standard drug. Inhibition of glucose uptake by yeast cells (%) was estimated by following formula;

$$\text{(\%) Inhibition of glucose uptake by eukaryotic cell} = \frac{A_S - A_C}{A_S} \times 100$$

Where,
As = Absorbance of sample and
Ac = Absorbance of control

c) Detection of α-Amylase inhibition using starch-iodine test

Starch iodine test was performed to check the α-Amylase inhibitory assay by using previously reported protocol (Fuwa, 1954). One ml of Starch solution (1% w/v), 1 ml of Acarbose solution/ plant extract with increasing concentrations (100, 200 mg/ml), 1 ml of α-amylase solution (1% w/v), and 2 ml of acetate buffer (0.1 M at 7.2 pH) was mixed. Iodine-iodide indicator (0.1 ml) was added in the mixture followed by incubation of 60 minutes. Absorbance of the mixture was measured by UV-Visible spectrophotometer adjusted at wavelength of 565 nm. Percentage inhibition was estimated by following formula;

$$\text{Percentage inhibition in amylase activity} = 100 - \frac{(E_{sample} - E_{control})}{100}$$

Where,
E sample = Absorbance of enzyme activity in sample and
E control = Absorbance of enzyme activity in control

d) Binding affinity of glucose to plant extract

The bioactive compound of plant extracts exhibit glucose binding affinity therefore the glucose binding capacity of methanolic plant extracts was determined by using the protocol reported by Kumaran and Karunakaran, 2007 with slight modifications. Glucose solution having different concentration (20mM, 50mM, 100mM) and plant extract (1%) were prepared in autoclaved water and DMSO respectively. One ml of both solutions were mixed in test tubes and stirred well. The glucose content in supernatant of mixture were determined before incubation and noted as G1 and after 6 hours incubation and noted as G6. The mixture was centrifuged at 3,000 ×g for 20 minutes to get supernatants. Then, the glucose content in the supernatant was measured colorimetrically by commercial glucose kit at 500 nm. Metronidazole was taken as standard. All experiments were performed in triplicate.

$$\text{Glucose bound} = \frac{G1 - G6}{\text{Weight of the sample}} \times \text{Vol of solution}$$

Where,
G1 = Glucose concentration of the original solution and
G6 = Glucose concentration after 6 hours

e) Glucose consumption by Lactic Acid Bacteria (LAB)

Several stains of LAB were used to evaluate their glucose consumption ability. Bacterial cells were cultured in de Man Rogosa Sharpe (MRS) broth media. The bacterial cells were prepared by washing with distilled water repeatedly followed by centrifugation at 10,000 revolution per minute (rpm) for 10 minutes. The turbidity of the bacterial solution was adjusted at Optical Density 1.00±0.02 corresponding to colony forming unit (CFU) 1011 cells/ml. Glucose solution (1 ml) and 0.1 ml of freshly prepared bacterial cells were incubated for 10 minutes at 37°C. The mixture was centrifuged at 3000 ×g for 5 minutes. Glucose contents were estimated in

supernatants calorimetrically at 400 nm by using UV-Visible spectrophotometer. Glucose utilized by the bacteria was estimated by following formula;

$$\text{Utilized glucose} = \text{Glucose conc.}_{\text{(before incubation)}} - \text{Glucose conc.}_{\text{(after incubation)}}$$

2.4. Statistical Analysis

All the experiments were performed in triplicates and data was analysed by using one way ANOVA followed by Duncan Multiple Range Test (DMRT) using SPSS 16.0 software. All the values were taken as mean ± S.E and p value ≤5 was considered as significant and graphs were plotted in Microsoft Office 2013.

3. Results

Inhibitory effects of various concentrations of plants extract were evaluated for non-enzymatic haemoglobin glycosylation. Among all plants *C. officinalis* showed the highest inhibitory activity (83.90 %, 91.74 % and 94.80 % at plant concentrations 0.57, 1.24, and 3.24 mg/ml respectively) while *Z. jujube* showed least inhibitory effect (23.51%, 38.1% and 43.97 % at plant concentrations 0.57, 1.24, and 3.24 respectively) when compared with Acarbose.

The rate of uptake of glucose was estimated by measuring the amount of remaining glucose in the medium after incubation period of 30 minutes. Plant extracts exhibited the inhibitory effect on uptake of glucose by yeast cells. All the plants showed significant inhibitory effect when compared with acarbose at all concentrations. Highest inhibitory effect was shown by *Z. jujube* (upto 91.73% at a concentration of 500 mg/ml) and least inhibitory effect was observed by *S. indica* (less than 60% at all concentrations).

The α-amylase inhibitory activity of MeOH extract of selected medicinal plants was evaluated and compared with standard drug Acarbose. *S. indica* (Leaves) extract showed the highest inhibition of α-amylase (95.85%) at both low and high concentration. Other plants also have significant inhibitory effect when compared with standard drug Acarbose. Among all plants *Z. jujuba* displayed least inhibitory effect (84%) on α-amylase activity.

Anti-diabetic potential of various probiotic strains was evaluated using in vitro glucose absorption capacity test. The capacity was evaluated by measuring the glucose left in the medium after incubation of cells in glucose mixture for 30 minutes. All the strains had significant glucose adsorption capacity except one strain showed in figure 4. Strain no. 2 which was *Enterococcus sp.* had the highest inhibitory effect (26.2%).

4. Discussion

Type-2 *Diabetes mellitus* has been treated by various methods including insulin, chemicals and herbal treatments. Various medicinal plants having anti-diabetic potential have been used to treat diabetes and its related complications (Modak *et al*., 2007). Among all plants used in traditional medicine almost 30% of the plants were biochemically and pharmacologically investigated (Osadebe *et al*., 2014).

The present study was designed to check the efficacy of locally available plants with therapeutic properties, probiotic strain. Methanolic extract of seven different plants and five strains of probiotics were investigated to check their efficacy by various biochemical tests. In previous studies various biochemical tests were reported (Gupta *et al*., 2013). Among these selected tests were performed including (a) non-enzymatic glycosylation of haemoglobin molecule (b) uptake of glucose by eukaryotic model (c) α-amylase inhibitory test using starch-iodine test and

(d) glucose absorption capacity of bacterial strains.

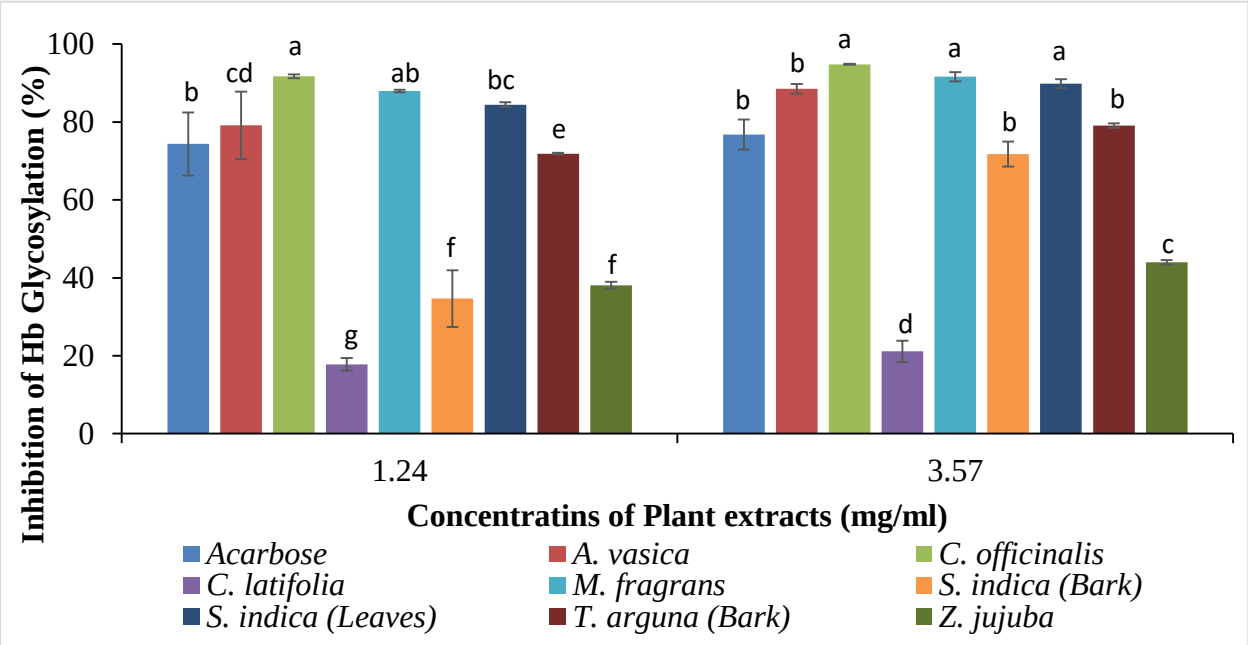

Figure 1: Effects of plant extracts/Standard drug on inhibition of hemoglobin glycosylation.

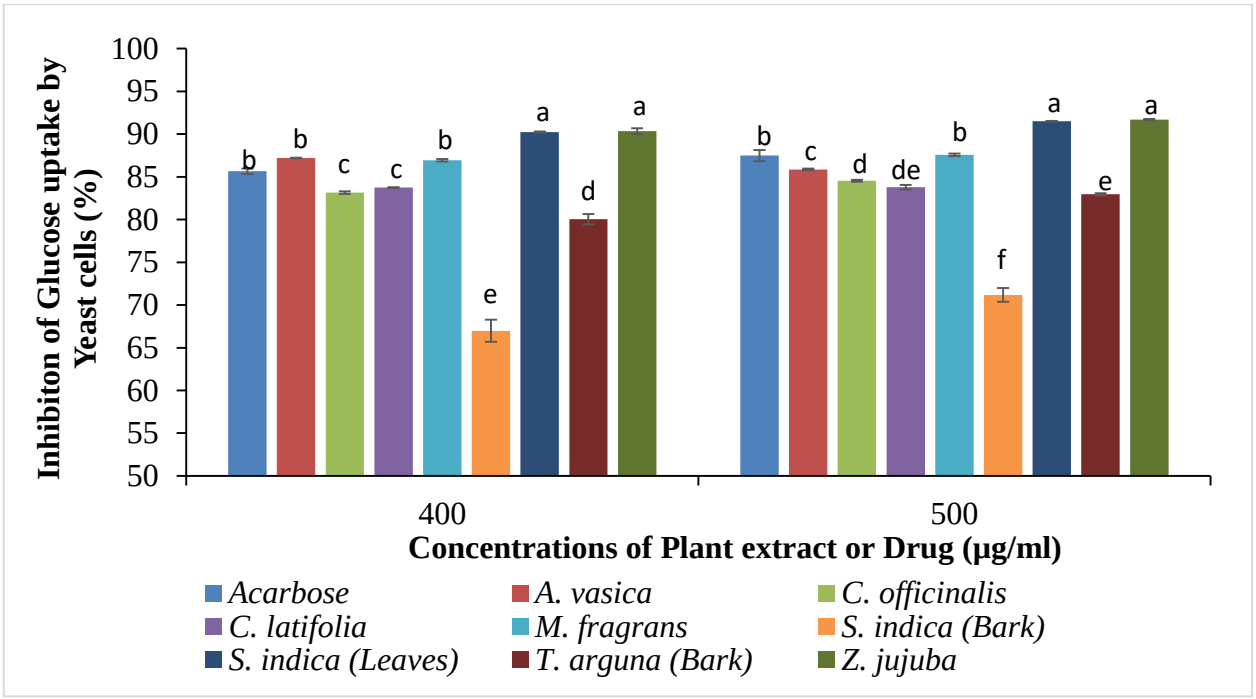

Figure 2: Effects of plant extracts and Standard drug on inhibition of glucose uptake by yeast cells.

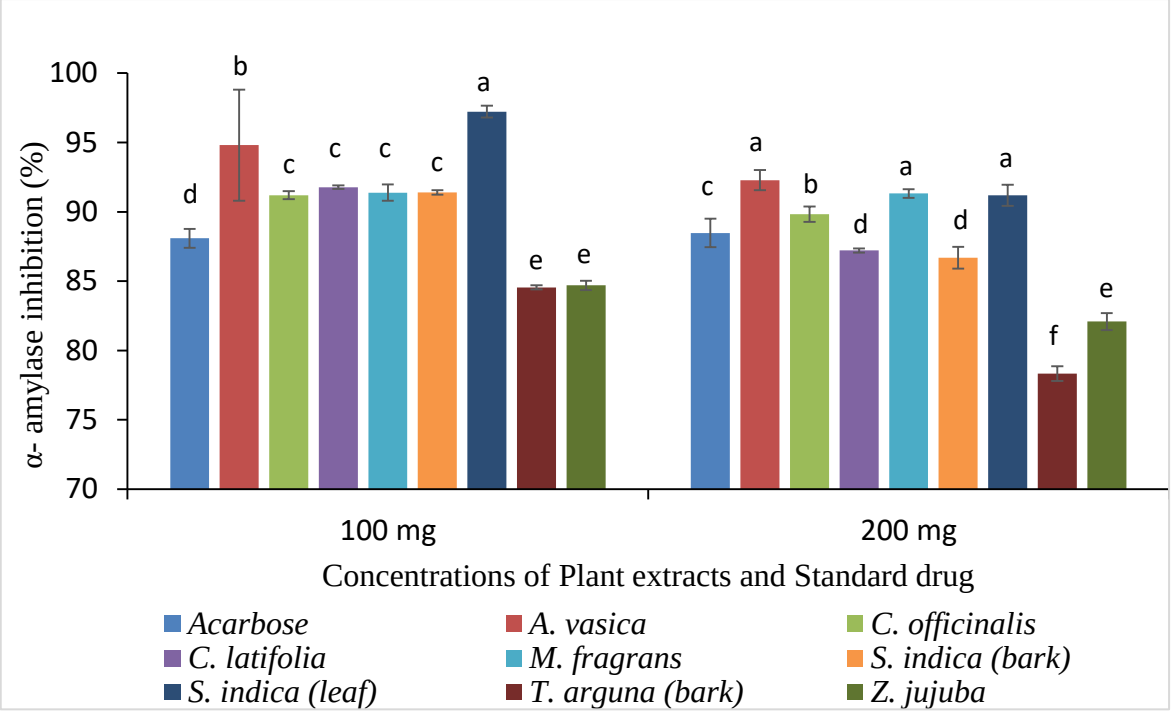

Figure 3: Effects of plant extracts on α-amylase inhibition using starch iodine test.

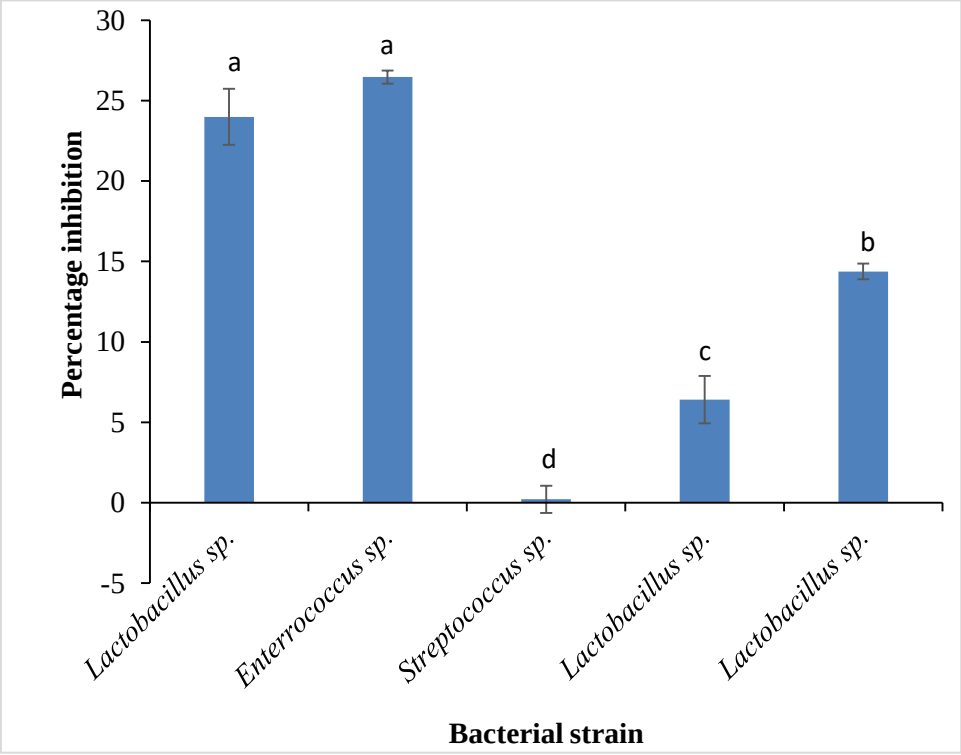

Figure 4: Effect of bacterial strain on percentage inhibition of glucose absorption.

Non-enzymatic glycosylation of haemoglobin test was used to confirm the status of diabetes (Chaudhari *et al*., 2013). If the amount of glycosylated haemoglobin exceeds more than 12 % it could be dangerous for vital functioning of the body Gupta *et al*. (2013) due to production of Reactive Oxygen Species (ROS) Non-enzymatic haemoglobin glycosylation may lead to the production of ROS. In previous studies various plant extracts exhibiting the inhibitory effect for glycosylation of haemoglobin molecule were reported (Gupta *et al*., 2013). Methanolic extracts of selected plants used in current study were selected for their potential to inhibit the haemoglobin glycosylation. *S. indica* was observed with highest inhibitory activity (89.84% at concentration of 3.56 mg/ml) for haemoglobin glycosylation.

Yeast was considered as model for in vitro test because of same working principal as the epithelial cells in digestive tract. Inhibition of glucose uptake by yeast cell is used as a screening method for hypoglycemic activity of various plant extracts and other bioactive compounds (Cirillo, 1962). In present study highest inhibitory activity for yeast cells were shown by leaves extract of *S. indica* (91.5% at concentration of 500 mg/ml).

α-amylase is an enzyme has been used to break down the glycosidic bonds of disaccharides and polysaccharides to produce glucose and its isomers. Acarbose has ability to inhibit the α-amylase activity but due to its side effects its use is limited so efforts had been done to discover the herbal extracts which may inhibit the function of this enzyme (Kazeem *et al*., 2013). In present study inhibition of α-amylase was checked by using MeOH extracts of various plants at different concentrations and results were compared with acarbose drug. The results showed the highest activity of *S. indica* leaves extract (95.09% at concentration of 200 mg/ml).

Glucose absorption capacity was investigated against five probiotic strains. The mechanism of action of bacteria is similar as the epithelial cells of intestine. All the bacterial strains had potential to absorb glucose from the solution but the *Enterococcus sp.* had shown the highest activity (26.2%). Literature survey showed no any significant article related to this test.

5. Conclusion

In this study anti-diabetic potential of medicinal plants (methanolic extracts) and lactic acid bacteria was evaluated. Among different parts of medicinal plants *C. officinalis* have promising effects in most of the biochemical tests. In vivo analysis is strongly recommended for future projections of this *C. officinalis* and Lactic acid Bacterial strain.

6. Acknowledgments

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7. Author's Contribution

Mohsin Munawar designed the whole study plane. Ayesha Shahid, M. Alam Sher and Mohsin Munawar conducted the experimental work. Technical assistance during the experimental work and proof reading of this manuscript was done by the Najma Arshad. Irfana Liaqat gave an idea and technical support to plan this work.

8. Conflict of Interest

Authors declared no conflict of interest.

9. Novelty Statement

Anti-diabetic potential of various medicinal plants was evaluated. We, in current studies, identified some regional plants having anti-hyperglycemic potential by using some in-vitro biochemical tests. In this study we

elaborate the anti-diabetic potential of lactic acid bacteria along with medicinal plants.

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