

Original Article

**PIPER CHABA EXTRACTS WITH ANTIBIOFILM ACTIVITY INFLUENCE ANTIHYPERGLYCEMIC AND ANTIHYPERLIPIDEMIC RESPONSES IN DIABETIC WISTER RATS**

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ABSTRACT

**Objectives:** *Piper chaba*, native to South and Southeast Asia, has been traditionally used as a medicinal plant. Aim of this study was to evaluate the antihyperglycemic and antihyperlipidemic activities of *P. chaba* root extracts (RE) in streptozotocin (STZ)-induced diabetic rats along with its antimicrobial activity.

**Methods:** Diabetes was induced in Wister rats through the intraperitoneal administration of STZ (50 mg/kg b.w.). Antidiabetic and antilipidemic activities of the RE (in methanol, ethanol, ethyl acetate and distilled water) were evaluated by administering oral dose (200 mg/kg b.w.) for 21 d. Metformin (12.1 mg/kg b.w.) was used as a positive control. Blood samples of rats were drawn by tail vein puncture and cardiac puncture to determine the fasting blood glucose (FBG) and serum level of total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL), respectively. Standard protocols were followed to determine the antimicrobial and antibiofilm activities against two different strains of bacteria.

**Results:** Oral administration of *P. chaba* RE for 21 d resulted in a significant ( $p < 0.001$ ) decrease in FBG and TC, TG, and LDL levels ( $p < 0.001$ ), when compared to the untreated diabetic rats. Significant ( $p < 0.001$ ) increase of HDL was observed when ethyl acetate and aqueous RE were administered. Out of four, two extracts showed varying antimicrobial activities, particularly against the gram-positive bacteria.

**Conclusion:** It became evident for the first time that *P. chaba* extracts possess antimicrobial activities and can serve as biochemical compounds with great alternative therapeutic potential in the management of diabetes and hypercholesterolemia.

**Keywords:** *Piper chaba*, Antidiabetic, Antilipidemic, Antibiofilm

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INTRODUCTION

In Bangladesh, four hundred and forty nine species of medicinal plants have been enlisted so far [1]. Among them, *Piper chaba* is a vine in the family of Piperaceae. It is found in the South Western region of Bangladesh, particularly at Jashore and Khulna regions, and people use it as an additive spice. Extracts of *P. chaba* possess phenolic compounds, antioxidants and showed anti-inflammatory, cytotoxic and anti-bacterial properties [2, 3]. Also, phytochemicals isolated from plants of the *Piper* species showed anticancer properties [4, 5].

Medicinal plants are of considerable interest to researchers in recent years to treat diabetes mellitus [6]. Diabetes mellitus (DM) is a group of chronic, complex metabolic disorder characterized by hyperglycemia due to defects in insulin production, insulin secretion, and insulin signaling [7]. Insulin is a potent hormone that regulates blood glucose level [8]. Globally, diabetes is the leading cause of human death. According to International Diabetes Federation (IDF), an estimated 463 million people, as of 2019, had diabetes worldwide (8.8% of the adult population), with type 2 diabetes making up about 90% of the cases [9]. According to the latest World Health Organization (WHO) data, DM-induced death reached to 28,065 or 3.61% of total deaths in Bangladesh [10]. Diabetes and hyperlipidemia are two factors responsible for the development of cardiovascular diseases. They have severe effect on lipid metabolism and show atypical effect on total lipid profile. Retinopathy, cardiovascular diseases, polyurea, polyphasia result from long time uncontrolled diabetes [11].

Besides insulin, the most widely used oral hypoglycemic drugs are Insulin sensitizer, insulin secretagogues,  $\alpha$ -glucosidase inhibitors, and dipeptidyl peptidase-4 inhibitors. But those drugs have remarkable toxicity and side effects [12]. On the contrary, the

traditional medicines prepared from herbal plants containing phenolic compounds having antidiabetic activity are cost effective with fewer side effects [13]. Also, WHO has been encouraging the use of traditional medicine to treat life threatening diabetes as well as its complications [14].

Plants of the Piperaceae family (such as *P. betle*, *P. crocatum*, *P. longum*, *P. nigrum* and *P. sarmentosum*) have been reported for their antidiabetic activities. Mixture extracts of *P. crocatum* leaves and *Cinnamomum burmannii* bark was reported to enhance the number of pancreatic  $\beta$  cells in rats, increase the rats' blood insulin levels, and reduce the rats' blood glucose levels [15]. Also, aqueous RE of *P. longum* was found to significantly decrease the hyperglycemic and hyperlipidemic activities in STZ-induced diabetic rats [16]. Additionally, in another study, aqueous extract of *P. nigrum* seeds and *Vinca rosea* flowers treatments lead to significant lowering of blood sugar level and reduction in serum lipids [17].

However, the literature review shows that there has been no study on the effects of *P. chaba* RE on STZ-induced rats to evaluate their antidiabetic and antihyperlipidemic activities. Hence, this study was undertaken to investigate these activities of *P. chaba* REs in induced diabetic rats.

MATERIALS AND METHODS

Collection of samples and preparation of extracts

Dry roots of *P. chaba* were purchased from the local market of Khulna, Bangladesh. Identification and authentication of the plant material were confirmed at the Department of Botany, University of Rajshahi, Bangladesh.

The sample was powdered using mortar and pestle. 250 gm of the powder was accurately weighed and separately dissolved with

methanol (MeOH), ethanol (EtOH), ethyl acetate (EtOAc) and distilled water (Aqueous, AQ), taking 1500 ml solvent and stirred for 72 h. It was then filtered and the filtrate was concentrated using a rotary evaporator (RE-401, Stuart equipment, Staffordshire, UK) at 30 °C-40 °C. The yield percentages were calculated using the following formula: Extract yield (%) =  $R/S \times 100$  (where R; weight of extracted plant residues and S; weight of the plant raw sample) [18]. The yield of EtOAc, MeOH, EtOH and AQ extracts were 28%, 12%, 20% and 25% w/w, respectively. The extracts were stored in small bottles at 4 °C.

#### Chemicals/reagents/diagnostic kits

The antidiabetic drug, metformin was obtained as a gift sample from Incepta Pharmaceuticals Ltd, Savar, Dhaka, Bangladesh. STZ (Sisco Research Laboratories Pvt. Ltd. India) and glucose standard strip/kits, and glucometer (GlucoXTD-4183 Blood glucose test strips, Germany). All other chemicals and reagents used were of analytical grade.

#### Experimental animals and ethical permission

Three-month old healthy male Wister albino rats (180-220 gm body weight) collected from the Pharmacology Lab, Department of Pharmacy, Jahangirnagar University, Dhaka, Bangladesh. This investigation was officially recognized by the Ethical Review Committee, Varendra University, Rajshahi, Bangladesh. Rats were kept in polypropylene cages group wise, 6 animals per cage. Before the experiment, the animals were synchronized to the experimental/laboratory condition for 7 d. The ambient temperature, humidity and 12 h day-night cycles were maintained according to the animal care and welfare guidelines [19]. All the animals were allowed to have a free access to fresh pellet diet and water.

#### Distribution of experimental animals in groups

For the evaluation of different extracts on the hyperglycemic and hyperlipidemic activities in normal and STZ-induced animals, adult male Wister rats were divided into 7 groups with six rats in each and treated with four different *P. chaba* extracts by gastric intubation via oral cavity:

Group-1: NDC-Non-diabetic control rats treated with standard diet

Group-2: DC-STZ-induced diabetic rats treated with standard diet (negative-control group)

Group-3: D+Metformin-Diabetic rats treated with standard diet and metformin (12.1 mg/kg b.w. in a day) (positive control group)

Group-4: D+EtOH-Diabetic rats treated with standard diet and ethanol extracts (200 mg/kg. b.w. in a day)

Group-5: D+MeOH-Diabetic rats treated with standard diet and methanol extracts (200 mg/kg. b.w. in a day)

Group-6: D+EtOAc-Diabetic rats treated with standard diet and ethyl acetate extracts (200 mg/kg. b.w. in a day)

Group-7: D+AQ-Diabetic rats treated with standard diet and aqueous extracts (200 mg/kg. b.w. in a day).

#### Induction of diabetes and test for antidiabetic study

A single dose of 50 mg/kg b.w. of STZ was prepared with 0.01M citrate buffer (pH 4.5). Then the freshly prepared solution was administered intraperitoneally to each experimental rat. After 48 h, rats with the fasting blood glucose (FBG) level of 200 mg/dl were selected as diabetic rats and were used for the study. The extracts were orally administered for 21 d once a day. On the 21<sup>st</sup> day, the blood sample was collected from the tail vein of rats and the FBG level was measured using one-touch glucometer (GlucoXTD-4183 Blood glucose test strips, Germany) with glucose oxidase-peroxidase reactive strips.

#### Measurement of serum lipid profile

At the beginning of the experiment, animals were deprived of food overnight. The animals were anaesthetized with chloroform and two milliliters of fasting blood samples was obtained from each animal of both the control and test groups via cardiac puncture. The blood sample was collected into a tube containing lithium heparin as

anticoagulant. The serum obtained after centrifugation at 3500 RPM for 15 min was separated for lipid measurement. The serum concentration of total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL)-cholesterol and low density lipoprotein (LDL)-cholesterol were measured using UV-spectrophotometric methods. Laboratory kit reagents (Human, Wiesbaden, Germany) following the company's instruction were used for all biochemical analysis and the absorbance was read using a UV-Vis spectrophotometer (Shimadzu UV-1280, Kyoto, Japan).

#### Formation of zone of inhibition in disc diffusion assay

Evaluation of the antimicrobial activity of all four *Piper chaba* root extracts was determined against *Bacillus cereus* (gram positive) and *Escherichia coli* (gram negative) by disc diffusion method [20]. Each bacterial strain was subcultured overnight at 37 °C in Mueller-Hilton agar slants. The bacteria were harvested using 5 ml of sterile saline water. Its absorbance was adjusted at 580 nm by a spectrophotometer and diluted to attain a viable cell count of  $10^7$  CFU/ml. Filter paper discs were loaded with *Piper chaba* stem extract at concentrations of 200 µg/disc and 400 µg/disc. The plates were kept in the fridge at 4 °C for 2 h. to permit the diffusion of plant extract. After incubating at 37 °C for 24 h, presence of inhibition zones were measured by a transparent scale in mm and recorded as well.

#### Determination of antibiofilm activity

Formation of biofilm biomass was determined through the microtiter plate assay in 96-well polystyrene flat bottom plates [20]. After overnight cultures of *E. coli*, optical density (OD) 1.0 was determined at 600 nm. 1% test microbes was mixed with 1 ml of immediately prepared LB media with or without the ethyl acetate and methanol extracts of *Piper chaba* roots (60-1000 µg/ml). After incubation at 37 °C for 24 h, plates were washed with distilled water to remove the free-floating cells. The biofilm was stained with 0.4% crystal violet (Himedia, Mumbai, India) solution. After 15 min, the staining process was complete and destaining process started with 1 ml of 95% ethanol. Finally, absorbance (at 650 nm) was measured by using a multiplate ELISA reader (Biotek-ELX-800, India). Formation of biofilm was determined by using the following equation:

$$\% \text{ of reduction of OD} = \frac{\text{Absorbance of (control-test)}}{\text{Absorbance of control}} \times 100$$

#### Statistical analysis

All data reported are presented as mean±SD or ±SEM. Statistical analysis was performed by using one-way analysis of variance (ANOVA) with multiple comparison tests, where applicable. The difference between two groups was measured by the student t-test. A P-value less than <0.05 was considered statistically significant.

## RESULTS

#### Determination of four different RE of *P. chaba* on FBG level in STZ-induced diabetic rats

The effect of EtOH-, MeOH-, EtOAc-, and AQ extract of the roots of *P. chaba* were tested on the FBG levels in non-diabetic and diabetic rats on Day 0 and Day 21 and are shown in fig. 1. The FBG levels of the STZ-induced diabetic control (DC, Gr-2) were significantly ( $p < 0.001$ ) higher than those of the respective non-diabetic control (NDC, Gr-1) tested both on Day 0 and Day 21 group of animals and the FBG level remained high in Gr-2 through Gr-7 tested on Day 0. However, as measured by one-way ANOVA, pretreatment of a single dose of 200 mg/kg b. w RE exhibited a significant hypoglycemic effect ( $p < 0.001$ ) tested on Day 21, when compared to DC. Metformin, a standard hypoglycemic drug, also had a significant ( $p < 0.001$ ) reduction in FBG level in Gr-3. Also, an intra-group analysis as measured by t-test, the FBG level revealed a significant ( $p < 0.001$ ) difference between Day 0 and Day 21 of each group (Gr-2 to Gr-7) (fig. 1).

#### Evaluation of different REs of *P. chaba* on lipid profiles in STZ-induced diabetic rats

Diabetes is also associated with altered lipid profile. The effect of EtOH-, MeOH-, EtOAc-, and AQ extract of the roots of *P. chaba* were tested on the serum levels of LDL, HDL, TC, and TG in STZ-induced diabetic Wister rats (fig. 2 and fig. 3).

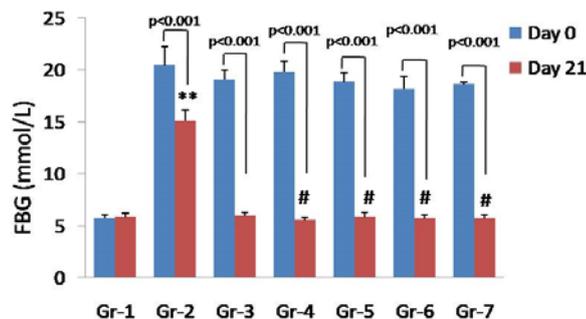


Fig. 1: Fasting blood glucose (FBG) level in normal and STZ-induced diabetic rats. n=5; Data are means±SD. \*\*p<0.001, versus respective control (Gr-1); #p<0.001, versus respective DC (Gr-2). The difference between the groups on day 21 was analyzed by one-way ANOVA followed by Bonferroni post hoc test and the difference between Day 0 and Day 21 of the same group was analyzed by t-test and the p value is shown inside the figure. Gr-1: non-diabetic control rats treated with standard diet (SD); Gr-2: STZ-induced diabetic rats treated with SD (negative control); Gr-3: Diabetic rats treated with SD and metformin (positive control); Gr-4: Diabetic rats treated with SD and ethanol extracts (200 mg/kg. b.w. in a day); Gr-5: Diabetic rats treated with SD and methanol extracts (200 mg/kg. b.w. in a day); Gr-6: Diabetic rats treated with SD and ethyl acetate extracts (200 mg/kg. b.w. in a day); Gr-7: Diabetic rats treated with SD and aqueous extracts (200 mg/kg. b.w. in a day). n= number of experimental animals.

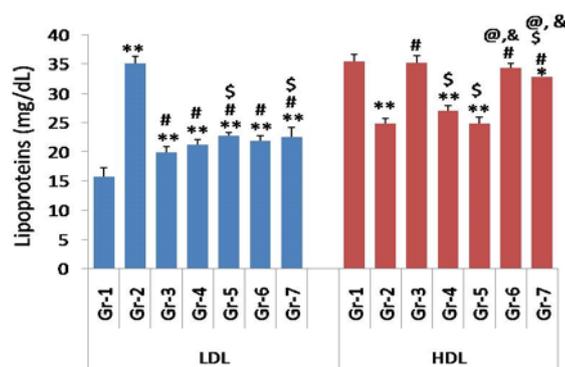


Fig. 2: Effect of *Piper chaba* root extracts on lipoproteins (LDL and HDL) in normal and STZ-induced diabetic rats after 20 d treatment. Values are presented as mean±standard deviation of the mean; n=6; p\* $<$ 0.05, \*\*p<0.001, versus Gr-1; #p<0.001, versus Gr-2; \$p = 0.002, versus Gr-3; #p<0.001 versus Gr-2; @p<0.001, versus Gr-4; and p<0.001, versus Gr-5. Gr-1: non-diabetic control rats treated with standard diet (SD); Gr-2: STZ-induced diabetic rats treated with SD (negative control); Gr-3: Diabetic rats treated with SD and metformin (positive control); Gr-4: Diabetic rats treated with SD and ethanol extracts (200 mg/kg. b.w. in a day); Gr-5: Diabetic rats treated with SD and methanol extracts (200 mg/kg. b.w. in a day); Gr-6: Diabetic rats treated with SD and ethyl acetate extracts (200 mg/kg. b.w. in a day); Gr-7: Diabetic rats treated with SD and aqueous extracts (200 mg/kg. b.w. in a day). n= number of experimental animals

The STZ-induced diabetic control (DC, Gr-2) significantly elevated the serum level of LDL (p<0.001), TC (p<0.001), and TG (p<0.001), but reduced the level of HDL (p<0.001), as compared to non-diabetic control (NDC, Gr-1, fig. 2). Metformin-treated diabetic rats (Gr-3) significantly reduced the levels of LDL (p<0.001), TC (p<0.001), and TG (p<0.001),

when compared with the DC. However, as compared to DC, metformin-treated diabetic rats (Gr-3) significantly (p<0.001) increased the level of HDL and showed no difference on the level of HDL, when compared with the NDC. Changes in serum lipids concentration following different RE-treatments were also evaluated as shown in fig. 2 and fig. 3.

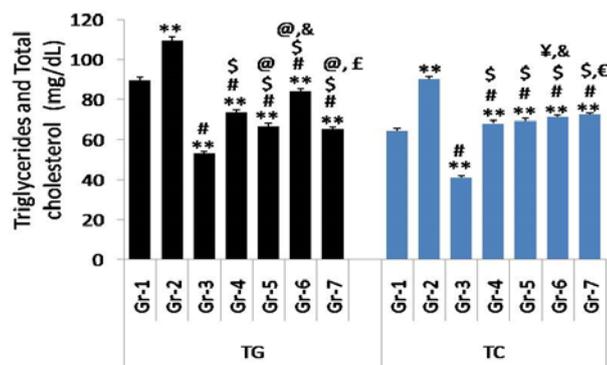


Fig. 3: Effect of *Piper chaba* root extracts on Lipoproteins (LDL and HDL) in normal and STZ-induced diabetic rats after 20 d treatment. Values are presented as mean±standard deviation of the mean; n=6; p\* $<$ 0.05, \*\*p<0.001, versus Gr-1; #p<0.001, versus Gr-2; #p<0.001 versus Gr-2; \$p<0.001, versus Gr-3; ¥p<0.01, @p<0.001, versus Gr-4; €p<0.01, and p<0.001, versus Gr-5; £p<0.001, versus Gr-6. Gr-1: non-diabetic control rats treated with standard diet (SD); Gr-2: STZ-induced diabetic rats treated with SD (negative control); Gr-3: Diabetic rats treated with SD and metformin (positive control); Gr-4: Diabetic rats treated with SD and ethanol extracts (200 mg/kg. b.w. in a day); Gr-5: Diabetic rats treated with SD and methanol extracts (200 mg/kg. b.w. in a day); Gr-6: Diabetic rats treated with SD and ethyl acetate extracts (200 mg/kg. b.w. in a day); Gr-7: Diabetic rats treated with SD and aqueous extracts (200 mg/kg. b.w. in a day). n= number of experimental animals

Treatments with extracts of EtOH, MeOH, EtOAc, and AQ extracts showed a significant ( $p < 0.001$ ) decrease in LDL, TC, and TG when compared with DC. On the contrary, EtOAc, and AQ extracts, but not EtOH, MeOH extracts, showed a significant ( $p < 0.001$ ) increase in HDL level, as compared with DC.

#### Determination of antimicrobial activity

Two extracts (methanol and ethyl acetate) represented varying antimicrobial activities in this study (table 1). The zone of inhibition (ZOI) against gram negative bacteria was bigger than that of gram positive bacteria. Susceptibility of these two extracts against bacteria increased with the increase in concentrations. Other two extracts (ethanol and aqueous) showed no antimicrobial activity.

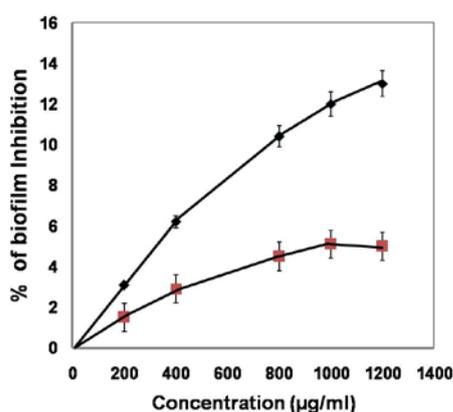
#### Effect of *Piper chaba* extracts on the formation of bacterial biofilm

Ethyl acetate and methanol extracts showed antimicrobial activity against *Escherichia coli* (table 1). Effect of these extracts on biofilm produced by the same bacteria was also checked. It was observed that ethyl acetate and methanol extracts dose-dependently inhibited the formation of biofilm when applied in increasing concentrations (60, 125, 250, 500, 1000 and 1200  $\mu\text{g/ml}$ ). SEM was calculated from three independent experiments. Comparing to the control, administration of ethyl acetate and methanol extract reduced the formation of biofilm from 3 to 13% and 2 to 5%, respectively. Percentage of inhibition became highest at a concentration of 1000  $\mu\text{g/ml}$  and become almost constant at 1200  $\mu\text{g/ml}$  (fig. 4).

**Table 1: Antibacterial activity of different extracts of the roots of *Piper chaba***

Diameter of zone of inhibition (mm)									
	Ethyl acetate ( $\mu\text{g/disc}$ )		Methanolic ( $\mu\text{g/disc}$ )		Ethanolic ( $\mu\text{g/disc}$ )		Aqueous ( $\mu\text{g/disc}$ )		Kanamycin ( $\mu\text{g/disc}$ )
Bacteria	200	400	200	400	200	400	200	400	30 $\pm$ 0.1
<i>Bacillus cereus</i>	09 $\pm$ 0.5	12 $\pm$ 0.43	08 $\pm$ 0.35	11 $\pm$ 0.6	Nil	Nil	Nil	Nil	29 $\pm$ 0.05
<i>Escherichia coli</i>	10 $\pm$ 0.31	15 $\pm$ 0.12	10 $\pm$ 0.25	14 $\pm$ 0.51	Nil	Nil	Nil	Nil	29 $\pm$ 0.07

Values are expressed as mean $\pm$ SD (n=3). n= number of experiments.



**Fig. 4: Antibiofilm activity of root extracts of *Piper chaba*. Black and orange boxes denote ethyl acetate and methanol extracts, respectively. Values are expressed as mean $\pm$ SEM; \* $P < 0.05$  was considered to be significant with respect to the control group**

#### DISCUSSION

The present investigation discusses the antidiabetic and antihyperlipidemic potential of the RE of *P. chaba* in STZ-induced diabetic rats. DM is a chronic metabolic disorder. Vascular complications are among the most common sequela of the condition, as cardiovascular disease accounts for 65% of mortality in DM [21]. Our study evaluated the effect of EtOH-, MeOH-, EtOAc-, and AQ extracts of the roots of *P. chaba* on STZ-induced diabetic rats. STZ, an N-acetyl glucosamine analogue, was used in our study to induce DM in rats. It destroys pancreatic  $\beta$ -cells by donating nitric oxide (NO), but has no effect on the exocrine part of pancreases [22].

Our short-term study confirmed the reducing effect of oral administration of *P. chaba* RE at a dose of 200 mg/kg b.w. on FBG level in diabetic rats significantly and the values of RE-treated rats after 21 d of treatment came to the level of the non-diabetic control rats. A similar outcome at the same dose after 30 d treatment of root aqueous extract was reported by *P. longum* [16]. The results of our study was also supported by the leaf extracts of *P. guineense* and *P. auritum* administered in diabetic female and male albino Wister rats, respectively [23, 24].

The recent studies have shown the increasing trends for the involvement of reactive oxygen species (ROS) and oxidative stress in the pathogenesis and development of type 2 DM [26]. A number of

important phytoconstituents such as dimeric alkaloids and alkamides have been isolated from various parts of *P. chaba* [27]. Earlier, many researchers have shown that *P. longum* and *P. chaba* extracts from different parts of the plant contain several phytochemicals and bioactive compounds like piperine, saponin, and sesquiterpene hydrocarbons  $\beta$ -caryophyllene,  $\alpha$ -humulene and germacrene D having their antioxidant properties [28, 29], that possibly scavenge free radicals too. On the other hands, the protein glycation and advanced glycation end products (AGEs) formation are associated with DM, which was also inhibited by the methanolic extract of *P. betle* and *P. auritum* leaves [30].

DM has been generally seen to have strong association with hyperlipidemia. The development of marked hyperlipidemia in diabetes may be a consequence of the uninhibited actions of lipolytic hormones on the fat depots and increase in mobilization of fatty acids from fat tissue [31]. In our investigation, we have observed the diabetic hyperlipidemia as marked with enhanced TG, TC, LDL, but decreased HDL cholesterol level. However, in STZ-induced diabetic rats, all four RE of *P. chaba* at a dose 200 mg/kg b.w. in our findings significantly reduced the level of TG, TC, and LDL, but increased the level of HDL when treated with EtOAc and AQ extracts. The LDL and HDL are considered as more particular and sensitive biochemical markers of cardiovascular disease. A thorough literature searches on hypolipidemic effect of the extracts of other species of the *Piper* genus also hold up our findings. In rabbits, the ethanolic fruit extract of *P. chaba* (150 mg/kg) possessed significant hypolipidemic effects and lowered TC and LDL levels while showing significant effect on HDL levels as well. Whereas, *P. nigrum* fruit extract (250 mg/kg) significantly elevated the levels of cholesterol and HDL and lowered LDL level on albino rabbits [32]. Also, other two different studies using the methanol and ethanol leaf extracts of *P. betel* at the dosages of 250 mg/kg b. w and 500 mg/kg b. w, respectively, on male albino Wister rats showed significant reduction of TC, TG, LDL and VLDL and HDL level became augmented [33]. It was suggested that the piperidine alkaloids including piperine, pipernonaline and dehydropipernonaline could be responsible for producing this effect by activating AMP-activated protein kinase that regulate lipid metabolism [34]. Our results following the same trend suggest that *P. chaba* REs can turn down the risk of diabetes-induced cardiovascular diseases by decreasing serum TC, TG and LDL levels and increasing serum HDL levels. The increased level of HDL is very significant due to its ability to reversely transport cholesterol from peripheral tissue and to esterifies cholesterol by lecithin-cholesterol acyltransferase (LCAT) [35]. So, the effect of *P. chaba* RE in diabetic disorder is remarkable.

Medicinal plants have been traditionally used for treatments against various bacteria. The antimicrobial activity of *piper chaba* root extracts showed dose-depend nature against *Bacillus cereus* (gram positive)

and *Escherichia coli* (gram negative). Our result showed the highest activity against gram negative bacteria and similar results have been found in previous studies [36]. It probably happened due to the presence of biologically active compounds such as phenolic, flavonoids, polyphenols, isoprenoids, alkaloids etc. in plant extracts and their effects on the cell wall components of bacteria. Structural and compositional difference in cell wall and cell membrane of gram positive and gram negative bacteria also plays a role [37-39]. An earlier study discovered a relationship between the antimicrobial activity and phytochemicals present in medicinal plants extract [40].

Our result suggested that the root extract was more active against gram negative microorganisms than gram positive microorganisms. *E. coli* are gram negative bacteria and have the ability to form biofilm [41, 42]. The antimicrobial activity of our plant (ethyl acetate and methanolic) extracts against gram negative bacteria encouraged us to investigate the properties of antibiofilm properties of these extracts. From our study it became evident that 200 µg/ml and 1000 µg/ml were the minimum and maximum concentrations of extracts to inhibit the formation of biofilm. Pronounced antibiofilm inhibition ability was found in ethyl acetate and also in methanol extracts. Similar result with solvent depended extracts of *P. longum* and *P. nigrum* were observed against *Streptococcus pyogenes* at the lowest concentration of 500 µg/ml [43]. Promising antibiofilm activity was observed in case of methanolic crude extract of *P. longum* against Vancomycin-resistant *S. aureus*-PAR1818 and *S. aureus* MH4 and MTCC 96 isolates whereas ethanol extracts of *Piper longum* fruits could reduce the cell attachment and inhibit the formation of biofilm by *Staphylococcus aureus* at 500 µg/ml [44, 45]. Walmiki *et al.*, found that *Piper nigrum* possessed antibiofilm activity against *Escherichia coli* and *Salmonella spp* [46].

## CONCLUSION

The present findings, for the first time, demonstrated that the RE of *P. chaba* is capable of exhibiting significant antihyperglycemic and antihyperlipidemic activities in STZ-induced diabetic rats. Moreover, the extract increased the level of HDL, a protective lipid compound, in diabetic condition. Therefore, our results give scientific support for the use of this plant in traditional medicine for the management of diabetes and its associated complications though further molecular studies are required to investigate the mechanism underlying the antihyperglycemic and antihyperlipidemic effect of *P. chaba* REs. Moreover, marked antimicrobial and antibiofilm activities of these extracts have also been observed.

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## AUTHORS CONTRIBUTIONS

SR did the majority of the study and wrote the manuscript. KK, UF and SoR were involved in experimental works and data analysis. RH provided laboratory support, supervised the study and improved the manuscript through careful review and helpful suggestions.

## CONFLICT OF INTERESTS

Authors declare no conflict of interest with respect to the research reported herein.

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