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Phytochemical Screening and Evaluation of Antioxidant, Antidiabetic, and Antimicrobial Potential of *Bergenia ligulata*

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ABSTRACT

Bergenia ligulata, locally known as "Pashanbheda," belongs to the family Saxifragaceae and is a perennial herb with a short and thick stem. This plant has numerous medicinal properties including antioxidant, antidiabetic, antibacterial, and anti-inflammatory properties. The qualitative and quantitative analysis shows that the rhizome of this plant contains various phytochemical constituents. Qualitative analysis shows that it contains phenols, flavonoids, tannins, terpenoids, alkaloids, steroids, carbohydrates, and glycosides. Thirty- two bioactive compounds were identified during GC-MS analysis. Dihydro-3-methylene-5-methyl-2-furanon, Stigmast-5-en-3-ol, 9-Octadecenamide(Z), 6,9-Octadecadienoic acid, methyl ester, 9,12-Octadecadienoic acid (Z, Z)-, methyl compounds have antioxidant, antibacterial and anti-inflammatory activities. The total phenol and flavonoid content from the quantitative analysis revealed a significant amount of phenol i.e., 67.1±0.02*6mg/g, and flavonoid content, i.e., 89.11±0.01* mg/g. In vitro, antioxidant activities were estimated by diphenyl-1-picrylhydrazyl assay showing the highest % inhibition of 47.24±0.06* for plant extract and 41.63±0.057* for standard respectively. The *in vitro*, antidiabetic activities were estimated by alpha-amylase inhibition assay showing the highest % inhibition of 67.14±0.05* at 250 µg/ ml for methanolic plant extract and 61.42±0.01* at 250 µg/ ml for standard and *in vitro* antibacterial potential were detected by using Agar well diffusion process. The anti-bacterial action was found maximum in aqueous plant extract against *staphylococcus aureus* bacteria.

Keywords: Antibacterial; Antidiabetics; Antioxidants; Flavonoids; Phenols; Phytochemical screening

1. INTRODUCTION

Medicinal plants are the only source of plant-based ethnomedicine and have been used since ancient times. All bioactive compounds produced by plants are collectively known as Phytochemicals. Medicinal plants contain many useful phytochemicals, especially phenols, flavonoids, terpenoids, steroids, alkaloids, tannins, saponins, and carbohydrates. These secondary metabolites show a definite physiological effect on the human body. Many chronic diseases such as diabetes, heart problems, and urinary problems can be protected by phytochemicals. Bergenin was isolated from the *Berginia* species and exhibited various biological effects such as hepatoprotetcive[1], antiarrhythmic[2], neuroprotective, antiviral[4], antibacterial[5], and anti-inflammatory[6]. Due to such an inclusive variety of biological actions of Bergenin, numerous studies[7,7]. have been dedicated either to a derivatizing molecule or synthesizing its interrelated compounds. A series of Bergenin derivatives with increased anti-oxidant potential was synthesized by many scientists.

Free radical-induced oxidative stress-induced damage was reduced by antioxidants[9]. The Free radicals generation is a fundamental feature of a normal cellular state, however, during pathological conditions generation of radicals increases manifold[10]. Free radicals can damage cellular proteins, lipids, and DNA and inhibit their function leading to metabolic imbalances which accelerate aging. Flavonoids have been investigated for their free radical-scavenging properties, which have allowed phenolic components of naturally occurring phytochemicals to be identified as antioxidants[12]. Most antioxidant activities are due to plant phenolics, found in all parts of medicinal plants[13].

Diabetes mellitus, a serious health problem that affects 185 million people, is also managed by secondary metabolites which having antidiabetic potential, particularly in developing countries where resources are limited[14]. The most commonly used therapies for diabetics' treatment include regular insulin injection and oral intake of antidiabetic medicine. These synthetic hypoglycemic medicines have adverse effects on human health like hepatic disorders, kidney tumors, and liver injury therefore effective antidiabetic medicinal plants are being explored. In the literature, approximately 450 therapeutic plant species have been reported that have hypoglycemic activity[14] and are used effectively to cure diabetes. The phytochemical constituents of medicinal plants also possess antibacterial activities. The various bioactive compounds analyzed by GC-MS analysis of *Berginia ligulata* are

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responsible for antimicrobial activity.

The methanolic extract of *Bergenia ligulata* used in our current study was evaluated for phytochemical screening and anti-oxidant, antidiabetics, and antimicrobial activities using standardized methods. The rhizome of *Berginia ligulata* was extracted with various organic solvents used for quantitative and qualitative analysis. *Bergenia ligulata* rhizome has been used for the treatment of kidney and bladder stones[15]. It is believed that the significant phytochemical qualities recognized by the present study will be extremely beneficial in the treatment of various illnesses.

2. METHODOLOGY

2.1 Plant Collection

The experimental plant was gathered from Shimla district of Himachal Pradesh (Figure 1). Identification of plant species was done by comprising the authenticated herbarium species present in Dehradun's Botanical Survey of India (BSI) North West Circle Herbarium and deposited (accession no. 468).

Figure 1. Berginia ligulata plant

2.2 Plant Extracts Preparation

To remove the impurity, the fresh plant parts were properly washed first under tap water and shade dried on a paper towel at room temperature. Using a grinder, dried rhizomes were ground into powder. To prepare the samples 5gm powder was diluted separately in 10ml of different solvents (methanol, ethanol, petroleum, and water) for 24 hours. After two days, the extract was filtered (Whatman paper 1). By using a rotary flash evaporator under reduced pressure, the whole solvent extracts were concentrated to dryness. The dried plant extracts obtain after rotary evaporation, were stored at -4 °C in an airtight bottle free from contamination. Finally, by using standard procedure, quantitative and qualitative analysis of plant extracts was carried out.

2.3 Qualitative Analysis

2.3.1 Molish Test for Carbohydrate

Two-three drops of alpha naphthol were added to two ml of aqueous extract of plant and then one mL conc. H_2SO_4 was added. At the junction, a violet ring appeared.

2.3.2 Alkaloids Test

Plant extract (2ml) was acidified with dilute hydrochloric acid, stirred well, and then filtered. The following tests were performed by using this filtrate[16].

2.3.2.1 Mayer Test

Mayer reagent drops were added to 3 milliliters of filtrate along the sides of the test tubes. White and creamy precipitates indicated alkaloids were present.

2.3.2.2 Hager Test

Hager reagent were added to a 2-3ml filtrate in the test tube. Yellow precipitate formation indicated alkaloids were present.

2.3.2.3 Wagner's Test

Wagner reagent (1-2 drops) was mixed with 1-2 ml filtrate. A reddish-brown precipitate indicated alkaloids were present.

2.3.3 Froth Test for Saponins

Distilled water (10ml) was mixed with 2gm of plant sample to dilute the sample, then boil it for 5 minutes. Then filtered the mixture. The filtrate (2.5 ml) was mixed with 10 ml of de-ionized water and vigorously shaken. Foam formation indicated that saponins were present.

2.3.4 Flavonoid Test by using Alkaline reagent test

Plant extract developed yellow color after adding a few drops of NaOH solution that become colorless when dilute acid was added. This indicates flavonoids are present in the plant[17].

2.3.5 Test for phenols

2.3.5.1 $FeCl_2$ Test

The plant extract was treated with 5% $FeCl_2$. The Green, violet, or blue colors are formed by the presence of phenolic compound[17].

2.3.5.2 Lead Acetate Test

The plant extract was mixed with 2-3 drops of lead acetate solution. Presence of phenolic compounds is specified by the formation of a white precipitate.

2.3.6 Salkowski Test for Steroids and Terpenoids

Chloroform was used to treat the plant extract, which was then filtered. To the filtrate, one or two drops of strong sulfuric acid were added, agitated, and left to stand. The lower red layer indicates the presence of sterols. The occurrence of steroids and terpenoids are

indicated by red and yellow gold layers on the lower side respectively[17].

2.4 Quantitative Analysis

Quantitative analysis was used to determine the phytochemicals of the plant extracts. We analyzed the phenolic and flavonoid content of *Berginia ligulata* rhizome extract by using standard methods.

2.4.1 Total phenolic content (TPC)

Folin- Ciocalteu protocol investigate the phenolic content present in the plant extract. The methanolic plant extract was prepared and added 10% FC reagent (2.5 ml) was added to each concentration from 50 to 250 µg/ml. After five minutes, 7.5% Na_2CO_3 (2.5 ml) was added and then, test tubes were properly shaken. To make the final volume 5 ml of deionized water was used. The reaction mixture was subjected to incubation for 35 minute. Absorption was measured at 760nm Total phenolic content was estimated by using a linear regression curve obtain from the standard gallic acid curve.

2.4.2 Total flavonoid content (TFC)

Aluminum Chloride ($AlCl_3$) colorimetric protocol investigated TFC[19]. The plant extract was prepared in methanol concentrations ranging from 50 to 250 µg/ml. Thereafter, 10% $AlCl_3$ (0.1ml) and potassium acetate (0.1ml) were added to the methanolic plant extract. To make the final volume of 3 ml 80% of methanol was added and the reaction mixture was subjected to incubation at 37°C. Absorption was evaluated at 420 nm. A linear regression curve of standard quercetin explicated TFC.

2.5 *In vitro* study of antioxidant activity

2.5.1 DPPH free radical scavenging assay

The free radical scavenging activity of plant extract was determined by using 2,2-diphenyl-1-picrylhydrazyl(DPPH). As a control, 0. 1 mM DPPH prepared in methanol was used. Each test tube contained 2.7 ml of 0.1 mM DPPH and 1mg/ml of plant extract in Dimethyl sulfoxide concentration ranging from 50 to 250 µg/ml. DPPH was added to them. Ascorbic acid was taken as standard. After proper mixing, the reaction mixture was allowed to stand at 25°C for 30 minutes and absorption was evaluated spectrophotometrically at 517 nm[20]. The % inhibition was as:

$$\% \text{ inhibition} = \left[(Ab\hat{} - Ab\hat{}\hat{}) / Ab\hat{} \times 100 \right]$$

Here Ab^ and Ab^^ are absorbance of the control and the test sample.

2.6 *In vitro* study of Anti-diabetic activity

2.6.1 Alpha-Amylase Inhibition Assay

This assay was utilized to determine antidiabetic activity[21]. DMSO (dimethyl sulfoxide) was used to prepare plant extract (1mg/ml) ranging conc. from 50 to 250 µg/mL. About 1% of starch solution (25ml) was prepared in phosphate buffer (pH 6.9) and heated for about 15 minutes with vigorous shaking for Solubilization. Deionized water was added to make the final volume 25 ml. Approximate 20 ml of 96 mM 3,5-dintrosalicyclic acid (DNSA) and sodium potassium tartrate in 8 ml of 2M sodium hydroxide were used to prepare color reagent and then, diluted with 12 ml distilled water. Also, 0.001gm alpha-amylase in 100 ml of sodium phosphate buffer (pH 6.9) containing 6.7mM sodium chloride was used to prepare an alpha-amylase solution. The starch solution (1ml) was added with constant shaking to the reaction mixture and incubated at 20°C for one hour. After adding 1 ml enzyme solution and incubating at 20°C for 3-4 minutes, a color reagent (1 ml) was added. Followed to be boiled in a hot water bath for about 7-8 min. and then placed on the ice-cool beaker. The procedure was followed for blank except plant extract was not added and the alpha-amylase was added after the color reagent. Absorbance was observed by using a spectrophotometer at 540 nm for both blank and test. Acarbose act as standard. The standard curve of maltose was used to compute a milligram of maltose liberated. The equation for the calculation of % inhibition is:

$$\% \text{ inhibition} = \left[(Ab\hat{} - Ab\hat{}\hat{}) / Ab\hat{} \times 100 \right]$$

where Ab^ and Ab^^ are the absorbance of the control and the test.

2.7 *In vitro* study of Anti-bacterial activity

2.7.1 Agar well diffusion method

Anti-bacterial potential of plant extract was investigated by Agar well diffusion method[22,23]. The agar medium was poured on sterilized Petri-disc. Bacterial culture was swabbed on the agar containing petriplates. Then, a 6 mm wells were made aseptically and plant extract was poured into these wells. Then, agar having petriplates were incubated for 24 hours at 37°C. Plant extract diffused in the agar medium and the zone of inhibition around each well was measured.

3. RESULTS

3.1 Qualitative analysis

Analysis of phytochemical *Bergenia ligulata* evaluates many bioactive constituents present that can be further used for manufacturing many herbal medicines. Aqueous, methanol, and ethanol extract contained all phytochemicals except petroleum ether (Table 1).

Table 1. List of phytochemicals present in rhizome extract of Berginia ligulata

Phytochemical constituents	Methanol Extract	Ethanol Extract	Petroleum Ether Extract	Aqueous Extract
Phenols	+	+	_	+
Flavonoids	+	+	_	+
Terpenoids	+	+	_	+

Saponins	+	+	_	+
Tannins	+	+	_	+
Carbohydrates	+	+	_	+
Alkaloids	+	+	_	_
Steroids	+	+	_	+

*(+) indicate presence, (-) indicate absent

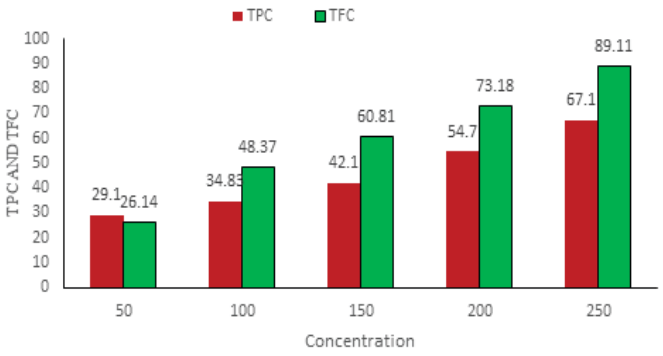
3.2 Total Phenol and Flavonoid Content

Total phenolic and flavonoids content were obtained from linear regression curve equation Y = 0.0031x - 0.0155 R^2=0.9835 and Y=0.0027x+0.6624 R^2 = 0.9933 respectively and expressed in term mg/g of gallic acid and mg/gm of quercetin. The phenolic and flavonoid contents were presented in the table & also expressed in the form of a histogram (Table 2 and Figure 2 & Figure 3).

Table 2. Estimated phenol and flavonoid content present in rhizome extract of *Bergenia ligulata*

The concentration of rhizome extract of *Bergenia ligulata* (µg/ml)	Total Phenol Content	Total Flavonoid Content
50	29.1±0.001*	26.14±0.03*
100	34.83±0.004*	48.37±0.02*
150	42.1±0.017*	60.81±0.03*
200	54.7±0.021*	73.18±0.02*
250	67.1±0.026*	89.11±0.01*

*Values represents mean ±SEM of three replicates



Values represent mean ±SEM of three replicates
Bars represent standard error at a 5% level

Figure 2. Showing TPC &TFC content of extract *Berginia ligulata*

3.3 Anti-oxidant Potential

3.3.1. DP-PH free-radical-scavenging-assay

Percentage inhibition of DPPH at a concentration 250 µg/ml, was found 47.24±0.06* for plant extract and 41.63±0.057* for ascorbic acid. The results show that plant extract act as a better antioxidant than standard ascorbic acid. The result of our present study at various concentrations is shown in Table 3 & Figure 4.

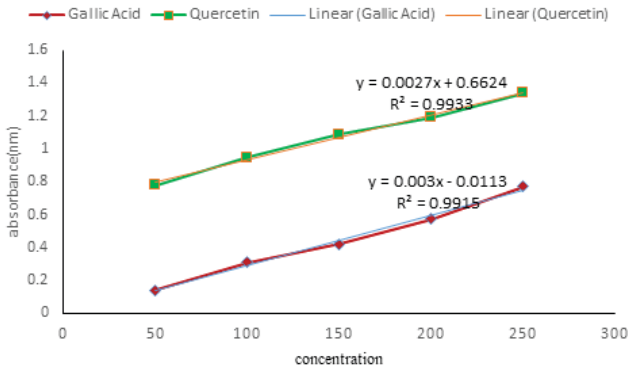
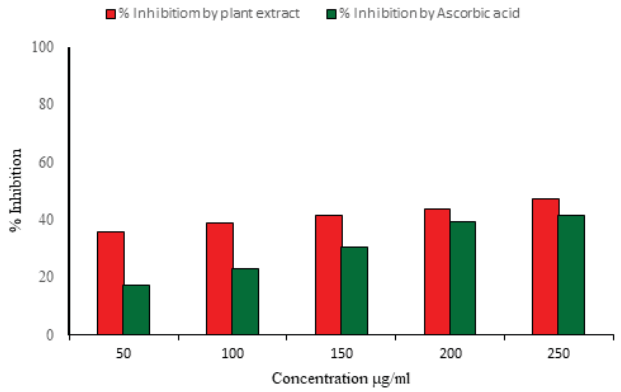


Figure 3. Gallic acid and Quercetin: standard curve

Table 3. Showing antioxidant activity of *Bergenia ligulata* extract

Conc. (µg/ml)	% inhibition of plant extract	Ascorbic acid % Inhibition
50	35.79±0.055*	17.38±0.155*
100	38.93±0.055*	23.22±0.150*
150	41.63±0.046*	30.4±0.152*
200	43.87±0.058*	39.38±0.10*
250	47.24±0.06*	41.63±0.057*

*Values- represents mean ±SEM of three replicates



Values represent mean ±SEM of three replicates
Bars represent standard error at a 5% level

Figure 4. Showing % inhibition of DPPH by *B. ligulata* extract and Ascorbic acid

3.4 *In vitro* Anti-diabetic Potential

3.4.1 Alpha-amylase inhibition assay

The percentage α-amylase inhibition potential for plant extract and acarbose was found 67.14± 0.05* and 61.42± 0.01* at 250 µg/mL respectively (Table 4 and Figure 6). The IC50 value for acarbose was 187.6 µg/ml, whereas, for the methanolic extract, IC 50 was

138.04 µg/ml. The standard curves for plant extract and acarbose are shown in Figure 5.

Table 4. Showing antidiabetic activity of methanolic rhizome extract of *Bergenia ligulata*

Concentration (µg/ml)	Plant extract % Inhibition	IC50 (µg/ml) of plant extract	Acarbose % Inhibition	IC50 (µg/ml) Acarbose
50	36.42±0.08*		20.54±0.02*	
100	44.28±0.03*	138.04 µg/ml	30.72±0.03*	187.6 µg/ml
150	51.48±0.07*		42.34±0.06*	
200	60±0.05*		55±0.01*	
250	67.14±0.05*		61.42±0.01*	

*Values represents mean ±SEM of three replicates

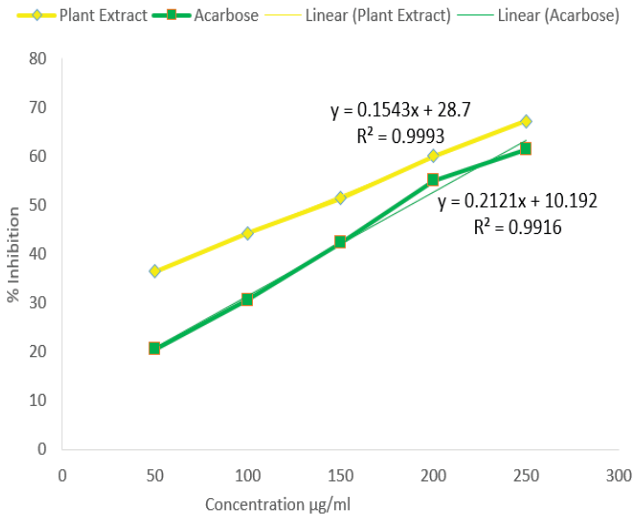
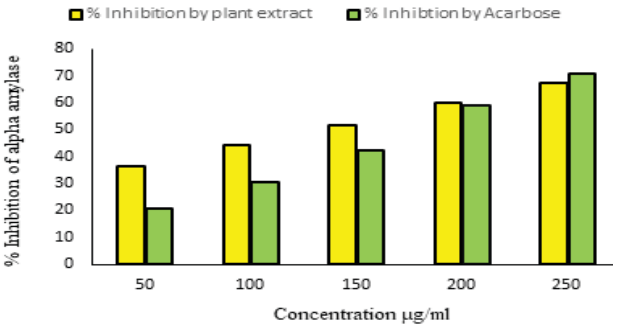


Figure 5. Standard curve for plant extract and acarbose

3.5 In vitro Study of Anti-bacterial Activity

3.5.1 Agar Well Diffusion Method

Antibacterial activity of aqueous and ethanolic plant extract was screened against the four bacteria out of which two are gram-positive and two are gram-negative. Results showed that plant extract was efficient enough to suppress bacterial growth. In the aqueous extract, a strong zone of inhibition of 12.07 mm was detected against *Staphylococcus aureus,* and in the ethanolic extract, maximum inhibition of 7.78mm was observed against *Bacillus subtilis* (Table 5 and Figure 7)



Values represent mean ±SEM of three replicates

Bars represent standard error at a 5% level

Figure 6. Differences in % inhibition of alpha-amylase by methanolic rhizome extract of *Bergenia ligulata* and Acarbose

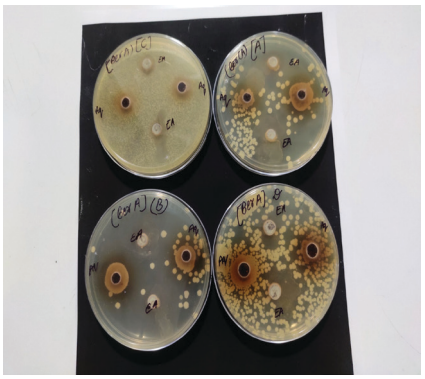


Figure 7. Antibacterial activity of aqueous and ethanol extract against bacteria A) *Staphylococcus aureus* B) *Salmonella abony* C) *Escherichia coli* D) *Bacillus subtilis*

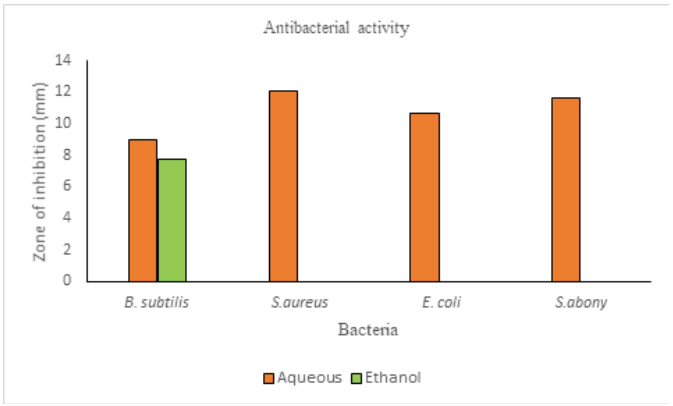


Figure 8. Zone of inhibition against different four bacterial strain

Table 5. Antibacterial inhibitory activity of rhizome extract of Bergenia ligulata against four bacterial strains

Sr. no.	Plant extract	Zone of inhibition of plant extract (mm)			
		Gram-positive Bacteria		Gram-negative Bacteria	
		Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella abony*
1.	Aqueous	09	12.07	10.64	11.60
2.	Ethanol	7.78	00	00	00

4. DISCUSSION

Herbal medicine is used by 70-80% of the population worldwide. Even though modern pharmaceuticals are extremely successful in curing sickness, herbal medicine, and treatments are becoming increasingly popular due to their fewer adverse effects. In the current study, the phytochemical contents found in the crude extracts of plants were examined for their effective health advantages, such as anti-diabetic, antioxidant, and antimicrobial activity. The plant extracts prepared in methanol show the presence of phenols, alkaloids, terpenoids, flavonoids, saponins, carbohydrates, or steroids. The phenols and flavonoids are associated with pharmaceutical and other medicinal properties due to their well-known high antioxidant activities. The plant-derived phenols and flavonoids are very effective and used as substitutes for synthetic antioxidants because the natural antioxidants are safer and have no adverse health effects[24,25].

The therapeutic effects are related to the phytochemical component of antioxidant activity[26]. Antioxidants are important for the control of human diseases because of their scavenging ability. The DPPH free radical scavenging method is an important method for determining plant extract antioxidant activity[27,28]. The presence of phenols, which can denote hydrogen atoms to the free radical, contributes to the DPPH radical scavenging activity[29]. Acarbose and other α-glucosidase inhibitors stop some enzymes in the small intestine from breaking down carbohydrates. Alpha-amylase is one of the key enzymes which break down starch into monosaccharides in the human small intestine. Diabetes is linked to oxidative stress, which is why phenolic chemicals have become a viable therapeutic option for diabetes. Many researchers have suggested that certain phytochemicals like cardiac glycosides[31], phenols, and flavonoids can inhibit alpha-amylase. The plant used in this study was discovered to have a massive amount of phenolic chemicals, containing 67.1 ±0.026*mg/g. As a result of redox potential, flavonoids reduced free radicals. Flavonoid content was found to be 89.11 ±0.01* mg/g. Methanolic rhizome extract had a DPPH free radical scavenging ability of 47.24 ± 0.06*, which was statistically significant and greater than their standard (Table -3). *S. aureus, B. subtilis, and K. pneumonia* were all inhibited by stigmasterol, 9 octadecenamide chemicals isolated from *B. ligulata*[30]. It has been established that the antibacterial properties of the genus *Berginia* are due to the presence of sterol and glycosides[31]. Our current findings also revealed a strong zone of inhibition against *B. subtilis.* The therapeutic potential of *Berginia ligulata* has been well elucidated in the present work.

5. CONCLUSION

The methanolic rhizome extract of *Berginia ligulata* was analyzed to check *in vitro* antioxidant, antibacterial, and antidiabetic activities. It has shown an inhibitory effect on alpha-amylase, therefore having an effective antidiabetic activity. According to several research, oxidative stress has a significant role in complications of diabetes[32]. The studied plant contained a large number of bioactive compounds like stigmasterol and 9-octadecenoic(Z)-, ethyl ester that was useful in the regulation of diabetes. Our finding provided scientific evidence that the bioactive compounds present in *Berginia ligulata* act as a good antioxidant, antidiabetic, and antibacterial agents.

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