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Research Article

# PHYTOCHEMICAL PROFILING AND *IN VITRO* ANTIDIABETIC AND ANTIOXIDANT ACTIVITIES OF *BETULA UTILIS* EXTRACT AND ITS FRACTIONS

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**ABSTRACT**

**Objective:** The study aimed to evaluate the phytochemical profile of *Betula utilis* stem bark extracts and assess their antioxidant and *in vitro* antidiabetic activities through α-amylase inhibition to support their traditional use in diabetes management.

**Methods:** Powdered stem bark was extracted using 80% ethanol and 80% methanol. The resulting crude extracts were further fractionated into diethyl ether, chloroform, and ethyl acetate fractions. Antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay. α-Amylase inhibitory activity was evaluated using the 3,5-dinitrosalicylic acid method. Extraction yields were calculated on a dry weight basis. Total phenolic and flavonoid contents were measured using the Folin–Ciocalteu and aluminum chloride colorimetric methods, respectively.

**Results:** The 80% methanolic extract demonstrated the highest antioxidant activity (55.40±1.62) and α-amylase inhibition (40.27±1.21), outperforming the 80% ethanol extract and individual fractions. Among fractions, the chloroform fraction exhibited the greatest α-amylase inhibition (23.30±0.68), while the diethyl ether fraction showed the lowest activity (7.57±0.21). The data suggest that moderately non-polar phytochemicals contribute significantly to enzyme inhibition, while the superior activity of the crude methanolic extract indicates synergistic interactions among bioactive constituents.

**Conclusion:** *B. utilis* stem bark contains bioactive compounds with promising antidiabetic potential. The 80% methanolic extract demonstrated notable α-amylase inhibitory and antioxidant activities *in vitro*, supporting its traditional use. However, cytotoxicity and safety evaluations are essential as the next step, followed by *in vivo* studies and isolation and characterization of active constituents to assess its therapeutic potential.

**Keywords:** *Betula utilis*, Antidiabetic, Antioxidant activity, α-amylase inhibition, Methanolic extract, 2,2-diphenyl-1-picrylhydrazyl assay, 3,5-dinitrosalicylic acid method.

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## INTRODUCTION

Diabetes mellitus, particularly Type 2 diabetes, has emerged as one of the most serious global health challenges of the 21st century. It is characterized by persistent hyperglycemia and associated metabolic disorders and predisposes individuals to complications such as cardiovascular diseases, neuropathy, and retinopathy, thereby increasing mortality [1,2]. Currently affecting approximately 460 million people worldwide, diabetes is among the fastest-growing non-communicable diseases. Projections suggest that this number may rise to nearly 700 million by 2045. The prevalence of diabetes is increasing rapidly, especially in low- and middle-income countries, where access to healthcare facilities, diagnostic tools, and effective treatments remains limited [3,4]. India is among the most affected countries, with recent large-scale studies reporting that over 100 million individuals are living with diabetes and an estimated adult prevalence of approximately 11–12% [5,6]. For every diagnosed case, several cases are likely to remain undetected, and the disease is increasingly being reported in younger populations. This alarming trend highlights the urgent need for safer, more accessible, and effective therapeutic strategies, while also posing a substantial economic burden on society. Consequently, there has been growing interest in phytochemicals and medicinal plants as alternative approaches for diabetes management [7,8].

For centuries, herbal medicines such as fenugreek, bitter melon, cinnamon, garlic, and neem have been used across various cultures to manage hyperglycemia. Recent reviews indicate that many medicinal plants exhibit antihyperglycemic effects through multiple mechanisms, including enhancement of insulin secretion, improvement of insulin sensitivity, antioxidant activity, and modulation of lipid metabolism [9,10]. Although several of these herbs have progressed to clinical evaluation, many others remain underexplored, particularly with respect to their phytochemical composition and fraction-wise biological activity [11].

*Betula utilis*, commonly known as Himalayan birch or Bhojapatra, is a tree native to the mountainous regions of the Himalayas [12]. Species of the genus *Betula* have a long history of traditional medicinal use, with bark and leaf preparations employed to treat inflammatory conditions, wounds, urinary disorders, and other ailments. Phytochemical investigations on *Betula* species have revealed a rich profile of triterpenoids, flavonoids, phenolic acids, and other metabolites with known antioxidant and enzyme-modulating properties. Although several *Betula* species (e.g., *Betula pendula, Betula platyphylla*) have been studied for their pharmacological activities, including antidiabetic enzyme inhibition and oxidative stress modulation, detailed studies specifically addressing the antidiabetic potential and underlying phytochemical mechanisms of *B. utilis* are limited [13-15]. The bark of *B. utilis* has been reported to contain sitosterol, betulin, betulic acid, oleanolic acid, acetyloleanolic acid, lupeol, lupenone, methyl betulonate, methyl betulate, and a novel triterpenoid, karachic acid [16]. Studies on related species, such as *B. pendula*, indicate that extracts can inhibit carbohydrate-hydrolyzing enzymes, including α-glucosidase, a mechanism directly relevant to postprandial glycemic control and commonly used in *in vitro* antidiabetic screening [17]. Taken together, the traditional use of *B. utilis*, its rich phytochemical composition,

and the limited antidiabetic studies on this species provide a strong rationale for investigating its *in vitro* enzyme inhibitory and antioxidant activities.

## METHODS

### Plant material

The stem bark of *B. utilis* was procured from Wholesale India Private Limited. The collected material was shade dried at 27±2°C for 7 days until a constant weight was achieved, and the final moisture content was determined to be 4%. The plant material was taxonomically identified and authenticated by Dr. Shrirang S. Bodke, Department of Botany and Horticulture, Yeshwant Mahavidyalaya, Nanded, India. A voucher specimen (Voucher No. H-2) was prepared and deposited in the Herbarium of the Department of Botany and Horticulture, Yeshwant Mahavidyalaya, Nanded, for future reference.

### Chemicals and reagents

Organic solvent used for the extraction of the plant material all purchased from Rankem. Alpha amylase enzyme and 3,5-dinitrosalicylic acid (DNSA) were procured from Sigma Aldrich, Bengaluru, India, and starch from Loba chemicals. All other chemicals used were of analytical grade.

### Physicochemical evaluation

Determination of the moisture content: The percent moisture content was calculated by the following formula:

$$\text{Percentage Moisture} = 1 - \frac{\text{Weight dry sample}}{\text{Weight wet sample}} \times 100$$

Determination of ash content: Percent ash was calculated by following formula:

$$\text{Percentage Ash} = \frac{\text{Difference in Weight of ash}}{\text{Weight of medicinal plant powder}} \times 100$$

### Determination of acid/alcohol/water soluble ash

The soluble ash was determined by using different solvents such as 5N HCl, alcohol, and distilled water. The ash obtained was digested with 25 mL of solvent for 20–30 min in a boiling water bath. The content in the crucible was filtered using ashless filter paper (Whatman filter paper No: 42). The filter paper with residue was removed carefully without any loss, folded, put in the same crucible, dried in a hot air oven, and ignited in a muffle furnace at 600°C for 1 h. Then it was cooled in a desiccator and weighed and the results are given in Table 1. The soluble ash value was calculated by the following formula.

$$\text{Percentage of soluble ash} = \frac{\text{Weight of soluble ash}}{\text{Total weight of ash}} \times 100$$

### Determination of phytochemicals

*Qualitative screening of phytochemicals*

A phytochemical screening of phenolics, alkaloids, flavonoids, saponins, tannins, terpenoids, steroids, cardiac glycosides, and glycosides/ reducing sugars present in plant extracts was performed using standard methods. Phenolics were determined as per the method given by Krishnaiah *et al*., 2009 [18]. Saponins indicate its presence as per methods given by Houghton and Raman, 1998, and Houghton and Raman, 2007 [19]. The terpenoids were screened by method of Trease and Evans, 2002. Cardiac glycosides were screened in the plant material by Krishnaiah *et al*., 2009 method.

### Quantitative phytochemical analysis

*Total phenolic content*

Principal

The Total phenolic content of the extract was determined by the Folin-Ciocalteu methodusing Gallic acid as a standard. This test is based on the oxidation of phenolic groups with phosphomolybdate and phosphotungstate. After oxidation, a green-blue complex formed, which measured at 760 nm.

Chemical required

Dilute Folin-Ciocalteu reagent by taking 10 mL to 20 mL with diluent 7% $Na_2CO_3$ Reagent, Diluent: Water.

Standard preparation: Dissolve 2.5 mg Gallic acid Reference Standard in 10 mL diluent.

Sample preparation: Transfer an accurately weighed 15 mg of the sample into a 10 mL volumetric flask, add about 80 mL of diluent, and heat on a water bath at 80°C for 60 min, cool, and dilute to volume with diluent and mix.

Procedure

Pipette out 1 mL from the standard solution and 1 mL from the sample solution in a separate 25 mL Volumetric flask, add 2 mL Folin-Ciocalteu reagent in each flask, mix well, add 11 mL of 7% $Na_2CO_3$ solution in each flask, and make up the volume with diluent. Shake well and allow the solution to stand in a dark place for 30 min. Measure absorbance after 30 min at 760 nm. Prepare blank as per the sample preparation method without adding sample. The percentage of total polyphenol content of the sample was determined using the following formulae. The results were expressed as mg of Gallic acid equivalent per g dry weight.

$$\textit{Total Polyphenol}\left(\%\frac{W}{W}\right) = \frac{At}{As} \times \frac{Wt.std}{10} \times \frac{1}{25} \times \frac{10}{Wt.spl} \times \frac{25}{1} \times \frac{P}{100} \times 100$$

Total Polyphenol (mg GAE/g) = Total polyphenol (%) × 10.

Where, $A_t$=Average absorbance of sample Solution, $A_s$=Average absorbance of standards Solution Wt. std.=Weight of standard in mg Wt. spl.=Weight of sample in mg.

P= Purity of working standard on as is basis.

*Total flavonoids content*

Principle

This method is based on the nitration of any aromatic ring bearing a catechol group with its 3–4 position unsubstituted or not sterically blocked. After addition of $AlCl_3$, a yellow solution of complex was formed, which then turned immediately to red after addition of NaOH, and the value of absorbance is measured at 533 nm.

Procedure

The total flavonoid content (TFC) of the extract was determined using aluminum chloride colorimetric method with quercetin as a standard. Briefly, the crude extracts (1 mg) were diluted with water (4 mL) in a 10 mL volumetric flask. Initially, 5% sodium nitrate solution (0.3 mL) was added to each volumetric flask then 10% aluminum chloride (0.3 mL) was added into the flask, followed by 1.0M NaOH (2 mL). Water (2.4 mL) was then added to the flask and mixed well. Absorbance of the mixture was read at 533 nm. TFC values were determined as quercetin equivalents mg/g of extract weight. All samples were analyzed in duplicate.

$$\textit{Total Flavanoids}\left(\%\frac{W}{W}\right) = \frac{At}{As} \times \frac{Wt.std}{10} \times \frac{1}{10} \times \frac{10}{Wt.spl} \times \frac{P}{100} \times 100$$

Total flavonoids (mg GAE/g)=Total flavonoïds (%)×10.

Where, $A_t$=Average absorbance of sample solution, $A_s$=Average absorbance of standards Solution Wt. std.=Weight of standard in mg Wt. spl.= Weight of sample in mg.

P=Potency of working standard on as is basis.

**Pharmacological screening of plant extracts**

*In vitro antioxidant activities of the plant extracts*

Free radical scavenging activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The free radical scavenging activities of the medicinal plants were determined by the DPPH method according to Brand-Williams *et al*. 1995 [20]. The scavenging activity was calculated by using the formula:

$$\text{Activity scravenging}(\%) = \frac{\left(\begin{array}{l}\text{Absorbance of the blank} - \\ \text{Absorbance of the sample}\end{array}\right)}{\text{Absorbance of the blank}} \times 100$$

The blank contained all reagents except the medicinal plant extract. Ascorbic acid at a concentration of 1 mg/ml was used as reference.

*In vitro method employed in anti-diabetic studies*

Inhibition assay for α-amylase activity

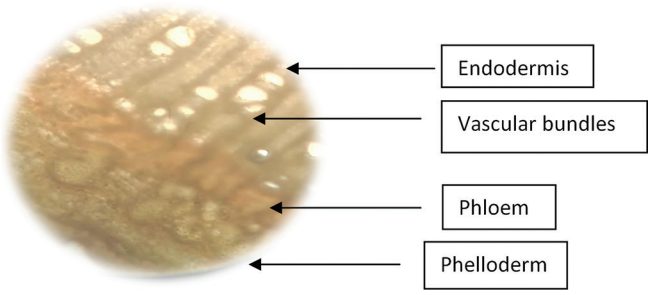
Plant extracts were dissolved in distilled water. A 100 µL of α-amylase (8U/mL) was mixed with the plant extract and incubated at 25°C for about 30 min. A 100 µL of this mixture was mixed with starch (0.5% w/v) solution (100 µL) and incubated at 25°C for 3 min. DNSA (DNSA reagent) (100 µL) was added incubated at 85°C for 15 min in a water bath, allowed to cool, and then diluted with distilled water (900 µL). Blanks were conducted in the same manner. Negative controls were prepared by adding DNSA reagent before the addition of starch solution kept in 85°C water bath for 15 min, and then diluted with distilled water (900 µL) as before. Absorbance was measured at 540 nm.

*Proximate composition of plant*

Stem bark microscopy

Section of stem bark stained with phloroglucinol HCl (1:1). The microscopic study of fresh stem bark section showed the presence of upper and lower epidermis, vascular bumdle, that is, xylem and phloem, mesophyll cells.

**Fig. 1: Thin-layer chromatography of the extract**




*Microscopical evaluation of powdered drug*

Some amount of powder drug stained with phloroglucinol HCL (1:1). It gives pink color to fibers and vascular bundles, which are observed

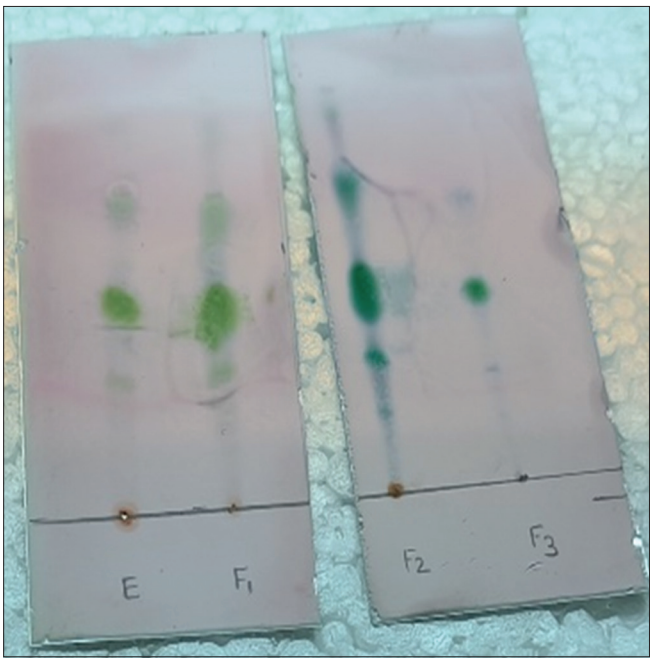



**Fig. 2: Thin-layer chromatography of the fraction**

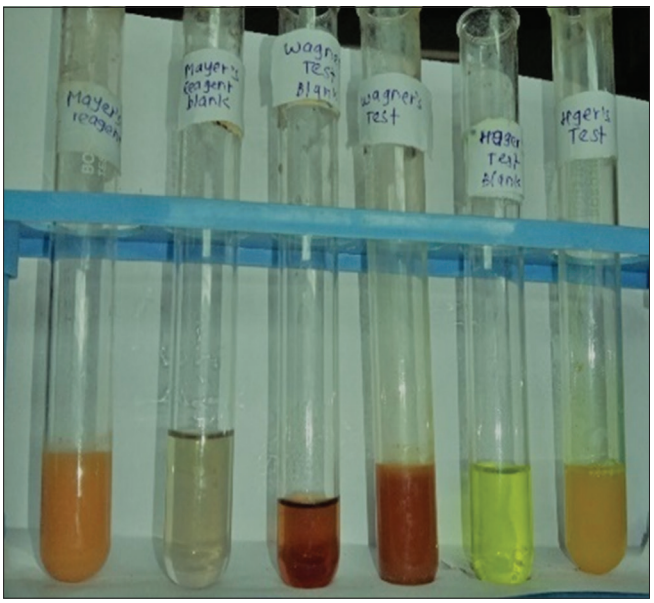
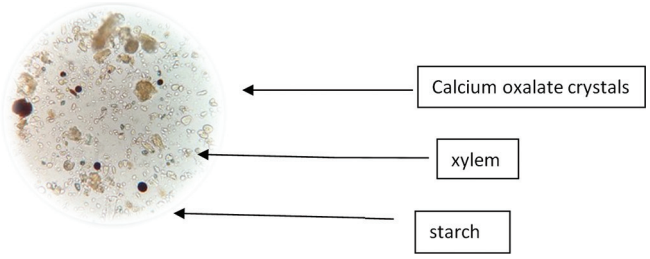



**Fig. 3: Chemical test of extracts**

under microscope with × 40 lens. The powdered drug showed presence of xylem, oligo resin crystals.




## RESULTS

### Statistical analysis

All experiments were performed in triplicate (n=3). Data are presented as mean±standard deviation (SD). Statistical comparisons between groups were performed using One-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. A $p<0.05$ was considered statistically significant. Analyses were conducted using GraphPad Instat version 5 (GraphPad Software, USA).

### Thin layer chromatography (TLC)

TLC profiles of *Betula utilis* extract and its fractions are presented in Fig. 1 and Fig. 2. The optimized chromatographic conditions are provided in Table 2, while the details of the extract tracks and sample tracks are given in Table 3.

**Table 1: Physicochemical parameters**

| Standardization parameter | Results (%) |
|---|---|
| Moisture content | 4 |
| Total ash (w/w) | 7 |
| Acid insoluble ash (w/w) (%) | 3 |
| Water soluble ash (w/w) (%) | 2 |
| Loss on drying | 5 |

**Table 2: Optimized chromatographic condition and track of extract**

| Parameter | Condition |
|---|---|
| Mobile phase | Methanol: Toluene (1.5:8.5) |
| Stationary phase | Precoated silica plates |
| UV wavelength | 366 nm |
| Diluent | Methanol |
| Concentration | 100 µg/mL |
| Derivatising agent | Anisaldehyde-sulphuric acid reagent |

UV: Ultraviolet

**Table 3: Sample track**

| Track | Sample |
|---|---|
| E | Extract BU 02 |
| F1 | BU_MF1 |
| F2 | BU_MF2 |
| F3 | BU_MF3 |

### Extraction of plant material

The plant material was subjected to different extraction protocols as summarized in Table 4. For most batches (BU02-BU05), the extraction involved direct extraction with either 80% methanol or 80% ethanol, using ultrasonication (45 min) followed by maceration (15 h) at room temperature. Soxhlet extraction was applied for BU03 using 80% methanol.

Batch BU01 differed from the other batches, as it included a defatting step with hexane before extraction with 80% methanol. This additional step was performed to remove non-polar constituents such as lipids and chlorophylls, which could interfere with subsequent extraction efficiency and bioactivity measurements. The defatting procedure introduces a methodological variation; therefore, BU01 should be interpreted separately when comparing yields and bioactivities with the other batches.

All extractions were conducted at room temperature with the solvent volumes indicated in Table 4. After extraction, the solvents were removed under reduced pressure, and the crude extract yields were recorded. The percentage yield was calculated relative to the initial dry weight of the raw material Table 4. BU stands for *B. utilis* extract.

### Phytochemical present in the plants stem bark extracts

The phytochemicals detected in the extracts of plants were flavonoids, phenols, alkaloids saponins, terpenoids, steroids, tannins, cardiac glycosides, and carbohydrate shown in Fig. 3 and Table 5.

The above observation table shows the presence of phytoconstituents in the *B. utilis* extracts. It reveals that most of the chemical constituent's present 80% methanol extracts and 80% Ethanol extract. Phytoconstituents such as alkaloids, flavonoids, phenols, and terpenoids present in all extract.

### Fractionation of methanolic extract

Based on its promising yield and bioactivity, the 80% methanolic crude extract (BU02) was subjected to further purification through sequential solvent partitioning to fractionate phytochemical constituents according to polarity. A 5.00 g portion of the dried crude extract was dissolved in 100 mL of distilled water to obtain the aqueous phase. Sequential liquid-liquid extraction was performed using diethyl ether, chloroform, and ethyl acetate.

Each extraction was repeated 3 times to maximize recovery. For each repetition, 50 mL of the respective solvent was added to the aqueous phase, and the mixture was vigorously shaken in a separatory funnel for 5 min. Complete phase separation was achieved by gravity at room temperature. The organic layers from each repetition were combined, dried over anhydrous sodium sulfate, and concentrated to dryness under reduced pressure at temperatures not exceeding 40°C to yield the fractions: BU_MF1 (diethyl ether), BU_MF2 (chloroform), and BU_MF3 (ethyl acetate).

**Table 4: Extraction condition and yield of different batches (mg/g dry weight)**

| Batch no. | Raw material (gram) | Solvents | Method of extraction | Time | Temp | Volume of solvent (mL) | Yield (g) | Percentage yield |
|---|---|---|---|---|---|---|---|---|
| BU01 | 5 | Defatting: Hexane and extraction: 80% methanol | Ultrasonication and maceration | 45 min and 15 h | Room Temp | 440 | 0.343 | 6.86 |
| BU02 | 5 | 80% methanol | Ultrasonication and maceration | 45 min and 15 h | Room Temp | 250 | 0.995 | 19.9 |
| BU03 | 5 | 80% methanol | Soxhlet | | Room Temp | 250 | 0.715 | 14.3 |
| BU04 | 5 | 80% ethanol | Ultrasonication and maceration | 45 min and 15 h | Room Temp | 250 | 0.936 | 18.72 |
| BU05 | 50 | 80% methanol | Ultrasonication and maceration | 45 min and 15 h | Room Temp | 5,000 | 12.09 | 24.19 |

**Table 5: Qualitative screening of the phytochemicals in the medicinal plants extracts**

| Chemical cons phytochemicals | Chemical test | BU01 | BU02 | BU03 | BU04 | BU05 |
|---|---|---|---|---|---|---|
| Alkaloids | Dragendorff's test | + | + | + | + | + |
| Glycosides | Borntrager's test | − | + | + | + | + |
| | Keller-killianin test | − | + | + | + | + |
| Phenols | Ferric chloride test | + | + | + | + | + |
| Carbohydrates | Molisch's test | + | + | + | + | + |
| | Barfoed's test | + | + | + | + | + |
| Saponins | Foam test | − | + | − | + | + |
| Terpenoids | Salkowski test | + | + | + | + | + |
| Flavonoids | Shinoda test | + | + | + | + | + |
| | Lead acetate | + | + | + | + | + |
| Steroids | Libermann-burchard test | − | + | − | − | + |
| Tannin | Ferric chloride test | − | + | + | + | + |

(Positive +, negative −)

**Table 6: Total phenolic content of *Betula utilis* extracts**

| Extracts | Sample absorbance | Standard absorbance | Percentage content | mgGAE/g mean±SD |
|---|---|---|---|---|
| BU01-Defat_80% methanol-UM | 0.612 | 0.743 | 13.5908 | 135.91±0.85 |
| BU02-80% methanol-UM | 0.757 | 0.743 | 16.8109 | 168.11±0.12 |
| BU03-80% methanol-reflux | 0.701 | 0.743 | 15.5673 | 155.67±0.45 |
| BU04-80% ethanol-M | 0.525 | 0.743 | 11.6588 | 116.58±0.98 |

SD: Standard deviation. Values are expressed as mean ± SD of three independent experiments (n=3). Statistical significance was evaluated using One-way ANOVA followed by Tukey's post hoc test ($p<0.05$)

**Table 7: Total phenolic content of 80% methanolic fractions**

| Fractions | Sample absorbance | Standard absorbance | Percentage content | mgGAE/g mean±SD |
|---|---|---|---|---|
| BU_MF1 (diethyl ether) | 0.214 | 0.708 | 4.9873 | 49.87±0.12 |
| BU_MF2 (chloroform) | 0.778 | 0.708 | 18.1314 | 181.31±0.75 |
| BU_MF3 (ethyl acetate) | 0.712 | 0.708 | 16.5932 | 165.93±0.98 |

SD: Standard deviation. Values are expressed as mean ± SD of three independent experiments (n=3). Statistical significance was evaluated using One-way ANOVA followed by Tukey's post hoc test ($p<0.05$)

**Table 8: Total flavonoid content of *Betula utilis* extracts**

| Extracts | Sample absorbance | Standard absorbance | Percentage content | Mg QE/g mean±SD |
|---|---|---|---|---|
| BU01-Defat_80% methanol_UM | 0.945 | 0.614 | 15.9282 | 159.28±1.15 |
| BU02-80% methanol_UM | 0.841 | 0.614 | 14.1753 | 141.75±1.02 |
| BU03-80% methanol_Reflux | 0.779 | 0.614 | 13.1302 | 131.30±0.98 |
| BU04-80% ethanol_M | 0.603 | 0.614 | 15.9282 | 101.63±1.18 |

SD: Standard deviation, QE: Quercetin equivalents. Values are expressed as mean ± SD of three independent experiments (n=3). Statistical significance was evaluated using One-way ANOVA followed by Tukey's post hoc test ($p<0.05$)

**Table 9: Total flavonoid content of 80% methanolic fractions**

| Fractions | Sample absorbance | Standard absorbance | Percentage content | MgQE/g mean±SD |
|---|---|---|---|---|
| BU_MF1 (diethyl ether) | 0.163 | 0.662 | 2.4958 | 24.96±1.12 |
| BU_MF2 (chloroform) | 0.871 | 0.662 | 12.9626 | 129.62±1.47 |
| BU_MF3 (ethyl acetate) | 0.766 | 0.662 | 11.0581 | 110.58±1.29 |

SD: Standard deviation, QE: Quercetin equivalents. Values are expressed as mean ± SD of three independent experiments (n=3). Statistical significance was evaluated using One-way ANOVA followed by Tukey's post hoc test ($p<0.05$)

The residual aqueous phase was discarded. All dried fractions were weighed, labeled, and stored at 4°C until further phytochemical analysis and bioactivity evaluation. TLC was performed to assess the chemical profiles of the fractions.

**Phytochemical quantitative analysis of extracts**

*Total phenolic content*

Methanolic extract shows more phenolic content than ethanol, as per the comparative evaluation of phenolic content of extracts. The Gallic acid was used as a reference Standard given in Table 6.

The 80% methanolic fractions of chloroform shows more phenolic content than diethyl ether and ethyl acetate as per the comparative evaluation of phenolic content of extracts. The Gallic acid was used as a reference Standard given in Table no 7.

*TFC*

Methanolic extract shows more flavonoid content than ethanol, as per the comparative evaluation of flavonoid content of extracts. The quercetin was used as a reference standard given in Table 8.

The 80% methanolic fractions of chloroform shows more Flavonoid content than diethyl ether and ethyl acetate as per the comparative evaluation of phenolic content of extracts. The Quercetine was used as a reference Standard given in Table 9.

*Free radical scavenging activity by DPPH assay of powders*

The antioxidant activity of plant extracts against DPPH radicals is primarily attributed to their hydrogen-donating capacity. Among the tested extracts, the 80% methanol extract of B. utilis exhibited the highest radical scavenging activity (55.40%), surpassing that of the 80% ethanol extract. given in Table 10. These results indicate that the stem bark extract possesses strong proton-donating ability and may function as an effective free radical scavenger, potentially acting as a primary antioxidant. Fractionation of the *B. utilis* extract revealed varying levels of radical scavenging activity. The chloroform fraction demonstrated the highest activity, 66.91%, followed by the ethyl acetate fraction, 63.62%, whereas the diethyl ether fraction exhibited the lowest activity, 15.59% given in Table 11. This suggests that the bioactive constituents responsible for antioxidant activity are predominantly concentrated in the chloroform and ethyl acetate fractions.

*Inhibition assay for a-amylase activity*

(DNSA): The results of the DNSA study are summarized in Table 12. All the extracts showed varying effect on glucose utilization. BU02 Extract: 80% methanol showed maximum inhibition of the enzyme with the highest value of 40.26% seen at 100 mg/mL concentration of plant extract given in Table 12.

Fractions obtained from the BU methanol extract were evaluated for in vitro α-amylase inhibitory activity. Among the tested fractions, the chloroform fraction exhibited the highest inhibitory effect (23.30%), whereas the diethyl ether and ethyl acetate fractions showed comparatively lower activities of 7.57% and 4.49%, respectively in Table 13. These findings suggest that the bioactive compounds responsible for α-amylase inhibition are predominantly concentrated in the chloroform fraction. Notably, the 80% methanolic extract displayed superior overall inhibitory activity relative to the individual fractions, underscoring its potential as a more effective antidiabetic agent.

## DISCUSSION

The present study demonstrates that *B. utilis* stem bark extracts possess significant antioxidant and α-amylase inhibitory activities, supporting their potential role in diabetes management. Phytochemical analysis revealed the presence of flavonoids, phenolics, and terpenoids, compounds known for their ability to reduce oxidative stress and modulate carbohydrate-metabolizing enzymes.

In the DPPH assay, the 80% methanolic extract exhibited the highest radical scavenging activity (55.40%), surpassing the 80% ethanol extract. Among the fractions, the chloroform (66.91%) and ethyl acetate (63.62%) fractions showed pronounced antioxidant activity, whereas the diethyl ether fraction displayed lower activity (15.59%). These results indicate that the extract possesses strong hydrogen-donating capacity, suggesting potential as a primary antioxidant capable of counteracting oxidative stress associated with diabetes.

The α-amylase inhibition study further corroborated the antidiabetic potential. The 80% methanolic extract demonstrated superior inhibitory activity relative to its fractions, highlighting the synergistic effects of multiple bioactive compounds. Among the fractions, the chloroform fraction exhibited the highest inhibition (23.30%), followed by the diethyl ether (7.57%) and ethyl acetate (4.49%) fractions, indicating that moderately non-polar compounds may play a significant role in enzyme inhibition, although their activity appears enhanced in the presence of the full phytochemical matrix of the crude extract. The DNSA assay confirmed these observations, with the 80% methanolic extract (*BU02*) achieving maximum inhibition of 40.26% at 100 mg/mL, this inhibitory effect may contribute to delayed starch digestion and reduced postprandial glucose release, a key therapeutic target in diabetes management.

Overall, the antidiabetic effects of *B. utilis* stem bark appear to result from the combined contribution of antioxidant activity and α-amylase inhibition. The higher bioactivity observed for the crude methanolic extract compared with the individual fractions indicates that multiple phytoconstituents may contribute collectively to the overall effect. This enhanced activity is consistent with complementary or additive interactions among bioactive compounds present in the whole extract.

**Table 10: Percentage free radical scavenging activity of *Betula utilis* extracts by DPPH assay**

| Extract | Sample absorbance | Control absorbance | Percentage inhibition, mean±SD |
|---|---|---|---|
| BU01-defat_80% methanol UM | 0.536 | 0.915 | 41.42±1.25 |
| BU02-80% methanol UM | 0.408 | 0.915 | 55.40±1.62 |
| BU03-80% methanol reflux | 0.414 | 0.915 | 54.75±1.58 |
| BU04-80% ethanol UM | 0.563 | 0.915 | 38.47±1.14 |

SD: Standard deviation, DPPH: 2,2-diphenyl-1-picrylhydrazyl. Values are expressed as mean ± SD of three independent experiments (n=3). Statistical significance was evaluated using One-way ANOVA followed by Tukey's post hoc test ($p<0.05$)

**Table 11: Percentage free radical scavenging activity of 80% methanolic fractions by DPPH assay**

| Extract | Sample absorbance | Control absorbance | Percentage inhibition, mean±SD |
|---|---|---|---|
| BU_MF1 (diethyl ether) | 0.796 | 0.943 | 15.59±1.02 |
| BU_MF2 (chloroform) | 0.312 | 0.943 | 66.91±1.78 |
| BU_MF3 (ethyl acetate) | 0.343 | 0.943 | 63.63±1.65 |

SD: Standard deviation, DPPH: 2,2-diphenyl-1-picrylhydrazyl. Values are expressed as mean ± SD of three independent experiments (n=3). Statistical significance was evaluated using One-way ANOVA followed by Tukey's post hoc test ($p<0.05$)

**Table 12: α-Amylase inhibitory activity of *Betula utilis* extracts**

| Extract | Sample absorbance | Control absorbance | Percentage inhibition |
|---|---|---|---|
| BU01-defat_80% methanol UM | 1.502 | 1.432 | No activity |
| BU02-80% methanol UM | 1.335 | 2.235 | 40.27±1.21 |
| BU03-80% methanol Reflux | 1.433 | 1.424 | No activity |
| BU04- 80% ethanol UM | 1.475 | 1.441 | No activity |

Values are expressed as mean ± SD of three independent experiments (n=3). Statistical significance was evaluated using One-way ANOVA followed by Tukey's post hoc test ($p<0.05$)

**Table 13: α-Amylase inhibitory activity of 80% methanolic fractions of *Betula utilis***

| Fraction | Sample absorbance | Control absorbance | α-Amylase inhibitory activity (% inhibition, mean±SD) |
|---|---|---|---|
| BU_MF1 (diethyl ether) | 1.44 | 1.558 | 7.57±0.21 |
| BU_MF2 (chloroform) | 1.195 | 1.558 | 23.30±0.68 |
| BU_MF3 (ethyl acetate) | 1.488 | 1.558 | 4.49±0.15 |

SD: Standard deviation. Values are expressed as mean ± SD of three independent experiments (n=3). Statistical significance was evaluated using One-way ANOVA followed by Tukey's post hoc test ($p<0.05$)

However, definitive evidence of synergistic interactions would require formal combination or additivity analyses, which were beyond the scope of the present study. Nonetheless, these findings underscore the potential advantage of using whole plant extracts, as they retain the natural complexity of phytochemicals that may enhance biological efficacy.

## CONCLUSION

The findings of this study demonstrate that *B. utilis* stem bark contains bioactive constituents with significant antidiabetic potential. The extracts were shown to modulate biochemical pathways associated with glucose metabolism and oxidative stress, providing experimental validation for their traditional use in diabetes management. These results highlight the 80% methanolic extract as a particularly promising source of synergistic phytochemicals.

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## AUTHOR CONTRIBUTIONS

All the authors have contributed equally from conceptualization to quality control of the document.

## CONFLICTS OF INTEREST

The author(s) do not have any conflicts of interest.

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