Exploring the ayurvedic drug: *Murva* for anti-diabetic potential

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**Abstract**

**Background:** *Bauhinia tomentosa* is a well-known medicinal plant considered under the Ayurvedic drug as “Murva”. *Murva* is used in the treatment various diseases such as intermittent fever, pain, etc., including hyperglycemia. **Objectives:** In continuation of our research, the present study reports the fingerprinting, anti-diabetic and pharmacognostical profile of *B. tomentosa* root. **Materials and Methods:** Aqueous and methanol extracts were screened for the acute toxicity studies, oral glucose tolerance test (GTT) and streptozotocin (STZ)-induced anti-diabetic screening. Diabetes was induced in Wistar rats by the single i.p. administration of STZ at the dose of 65 mg/kg and was confirmed after 48 h of STZ administration. Blood glucose levels were monitored in normal and diabetic rats at regular intervals of 0, 7, 14, and 21 days throughout treatment duration. Other parameters such as body weight, food and water intake, cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very LDL (VLDL) were also be evaluated. Thin layer chromatography (TLC) and high-performance TLC (HPTLC) studies were carried out on extracts to identify the major phytoconstituents. Pharmacognostical analysis of root powder was performed by microscopic, physicochemical, and preliminary phytochemical examination. **Results:** Acute toxicity studies of extracts showed that both (methanol, aqueous) the extracts were found to be safe up to 3000 mg/kg bw. Methanol and aqueous extract were found to possess statistically significant (P < 0.001) decrease in fasting blood glucose at high dose (400 mg/kg) after 90 min of treatment, whereas low doses (200 mg/kg) of both the extracts were failed to show the statistically significant effect in oral GTT. Treatment of STZ-induced diabetic rats with the methanol and aqueous extract (200, 400 mg/kg) showed statistically significant (P < 0.001) reduction in fasting BGL of the diabetic rats on prolonged treatment when compared with that of control as well as the reference drug, glibenclamide. Fingerprinting analysis of the extracts revealed the presence of 7-hydroxy flavones and marmesin and flavonoid 2 with the Rf corresponding to 0.42, 0.49, and 0.55, respectively may be responsible for the observed anti-diabetic effect. **Conclusion:** *Murva* is used as an efficient medication for the treatment of various diseases in Ayurvedic system of medicine. The present study reestablishes the plant as the effective medication for the management of diabetes. Further, HPTLC analysis showed the presence of important phytoconstituents, which are effective anti-oxidant, proves that it may be beneficial for the prevention of macro- and micro-vascular complications of chronic disease diabetes. Although, more advanced studies are required for elucidating the mechanism of action as well ensuring its effect in higher animals.

**Key words:** Anti-diabetic, anti-oxidant, fingerprinting analysis, glibenclamide, oral glucose tolerance test, streptozotocin

**INTRODUCTION**

Diabetes mellitus (DM) is becoming a global challenge in the 21st century. Every year, over 4 million people die from diabetes, and tens of millions will more suffer disabling and life-threatening complications such as heart attack, stroke, kidney failure, blindness, and amputation. Diabetes is also implicated in and has
negative consequences for certain infectious diseases, other non-communicable diseases, and mental health.[1]

Already, 366 million people have diabetes, and another 280 million are at identifiably high risk of developing diabetes. If nothing is done, by 2030 this number is expected to rise to 552 million with diabetes and an additional 398 million people at high risk. Three out of four people with diabetes now live in low- and middle-income countries. Over the next 20 years, Africa, Middle East, and South-East Asia regions will shoulder the greatest increase in diabetes prevalence. “No country, rich or poor, is immune to the epidemic.”[2]

Diabetes is not only a health crisis; it is a global societal catastrophe. Governments worldwide are struggling to meet the cost of diabetes care. Costs to employers and national economies are escalating, and every day low-income families are being driven into poverty by loss of earnings due to diabetes and the life-long costs of healthcare.

Plants are the natural gift, and various effective medicinal plants are available free of cost to the society, one of them is *Bauhinia tomentosa* (Figure 1). This plant is mentioned as an alternate source of the Ayurvedic drug *Murva*. Traditionally, this plant is used in numerous formulations for the treatment of intermittent fever, abdominal disturbances, urinary tract infection diseases, DM, convulsions, etc. Flower and stem of the plant have been reported to possess significant anti-diabetic effect. Therefore, the present study has been undertaken to evaluate the antihyperglycemic effect of *B. tomentosa* root.[3-6]

**MATERIALS AND METHODS**

**Chemicals**

Streptozotocin (STZ) was obtained from Sigma-Aldrich Co., St. Louis, USA. Solvents were purchased from Chemsynth Laboratories Pvt. Ltd., India. All the chemicals were used of analytical grade, whereas other biochemical kits were obtained from span diagnostic Ltd. India. Other chemicals of analytical grade used in the study have been obtained from the laboratory of Devsthali Vidyapeeth College of Pharmacy.

**Plant Material**

*B. tomentosa* roots were collected in September 2011 from the nursery of Rudrapur (Uttarakhand). Plant was identified and authenticated by the National botanical research institute (NBRI) Lucknow. The herbarium of the plant was prepared and deposited in herbarium of Devsthali Vidyapeeth College of Pharmacy (Varsha no. 10).

**Preparation of Extracts**

The shade-dried root powder (100 g) was extracted exhaustively with 70% v/v methanol in a soxhlet apparatus by continuous heat extraction. The methanol extract was concentrated to small volume and then evaporated to dryness. The methanol extract for the experimental purpose was prepared in distilled water containing 2% v/v tween 80 (as suspending agent).

Aqueous extract was prepared by maceration with chloroform water, followed by filtration and concentrating the extract to small volume and then evaporated to dryness. The aqueous extract for the experimental purpose was prepared in distilled water containing 2% v/v tween 80 (as suspending agent).

**Microscopic Studies**

It includes the transverse section studies and powder characteristic of plant to establish the diagnostic characteristics of plant.[7,8]

**Physicochemical Studies**

It includes the evaluation of physicochemical parameters such as moisture content, ash value, extractive value, and fluorescence studies.

**Preliminary Phytochemical Studies**

Phytochemical studies include the detection of major class phytoconstituents and then selecting the mobile phases for the chromatographic analysis.[9]

**Chromatographic Studies**

Thin layer chromatography (TLC) of extracts was performed in different mobile phases to select the best mobile phases for
the Fingerprinting analysis. Four best chosen mobile phases are:

- Mobile Phase I: CHCl$_3$: C$_2$H$_5$OH [9.8:0.2]
- Mobile Phase II: CHCl$_3$: CH$_3$OH [95:5]
- Mobile Phase III: Toluene: Ethyl acetate: Glacial acetic acid [5:7:1]
- Mobile Phase IV: Toluene: Ethyl acetate: Glacial acetic acid [6:4:1]

Thereafter, high-performance TLC (HPTLC) of extracts was carried out for identifying the major phytoconstituents.

**Pharmacological Activities**

**Experimental animals**

For the experiment male, Wistar rats weighing between 200-230 g were collected from the animal research branch of the IVRI Bareilly, Uttar Pradesh, India. Animals were maintained under standard environmental conditions (temperature: (24.0±1.0°), relative humidity: 55-65% and 12 h light/12 h dark cycle) and had free access to feed and water ad libitum. The animals were acclimatized to laboratory condition for 1 week before experiments. All protocols for the animal experiment were approved by the institutional animal ethical committee (CPCSEA/IAEC/2010-11/06).

**Acute toxicity studies**

Studies have been performed in previous experiments.[14]

**Anti-diabetic activity**

a. Oral glucose tolerance test (OGTT)

Overnight fasted rats were divided into six groups containing six animals each group. Experiment was designed as:

- Group-I was kept as control which received 5% tween 80
- Group-II received methanol extract (200 mg/kg)
- Group-III received methanol extract (400 mg/kg)
- Group-V received aqueous extract (200 mg/kg)
- Group-VI received aqueous extract (400 mg/kg)

Groups III-VI were loaded with glucose (2 g/kg p.o.) after 30 min of drug administration. Blood samples were collected from puncturing the retro-orbital sinus just before drug administration and at 30, 60, 90, 120 min after loading glucose. Serum glucose level was measured immediately using glucose estimation kit (Span Diagnostic Pvt. Ltd. Surat India).[10]

b. Induction of diabetes in rats

Diabetes was induced by a single intraperitoneal injection of freshly prepared STZ (65 mg/kg) in 0.1 M citrate buffer (pH 4.5) to overnight fasted rats. After 48 h of STZ administration, blood was withdrawn from the retro-orbital plexus, and plasma was separated by centrifugation and blood glucose levels were measured immediately by using glucose oxidase/peroxidase method to confirm diabetes.

(c) Experimental design

(d) Assessment of anti-diabetic activity in STZ induced diabetic rats

Diabetic rats were divided into 7 groups containing 6 animals each for assessment of anti-diabetic activity.

- Group I: Normal Control received food and water
- Group II: Diabetic Control, received 0.5 ml of 5% tween 80
- Group III: Standard received Glibenclamide (100 µg/kg)
- Groups IV and V: Aqueous extract treated group 200 and 400 mg/kg
- Groups VI and VII: Methanol extract treated group 200 and 400 mg/kg.

The treatment was continued daily for 21 days. Blood samples were collected from the retro-orbital sinus of overnight fasted animals, just before drug administration for glucose estimation on days 0, 7th, 14th, and 21st of the experiment.[11,12]

**Statistical Analysis**

The data were expressed as mean±standard deviation. Statistical comparisons were performed using two-way analysis of variance followed by Duncan’s multiple range test. The results were considered statistically significant if the P values were 0.05 or less.

**Fingerprinting Analysis**

Chromatographic studies were carried out following Wagner (1996) and Krebs (2005).[13,14]

In the present work, Camag HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 with 12 bit CCD camera for photo documentation, controlled by Win CATS-4 software were used. All the solvents used were of HPTLC grade obtained from MERCK. All weighing were done on Precisa XB 12A digital balance.

a. Preparation of extract

Methanol extract for analysis was prepared by dissolving the extract in methanol, and after sonication, the solution obtained used for the analysis. Aqueous extract for analysis was prepared by dissolving the extract in water then sonicated, centrifuged and the supernatant was used for the sampling.

b. Preparation of sample

Methanol extract was dissolved in methanol and sonicated, solution obtained was used for the analysis. Aqueous extract was dissolved in water and centrifuged supernatant obtained was used for the analysis.
c. Mobile phases
   Mobile Phase I: CHCl₃: C₂H₅OH [9.8:0.2]
   Mobile Phase II: CHCl₃: CH₃OH [95:5]
   Mobile Phase III: Toluene: Ethyl acetate: Glacial acetic acid [5:7:1]
   Mobile Phase IV: Toluene: Ethyl acetate: Glacial acetic acid [6:4:1]
d. Chamber used for mobile phase
   Camag twin trough chamber (10 × 10 cm)
e. Chamber saturation
   Chamber saturation was done for 18 h.
f. Stationary phase
   HPTLC plates silica gel 60 F 254 manufactured by E. MERCK K GaA (5 × 10 cm) were used as stationary phase.
g. Procedure
   The samples of aqueous and alcohol extracts were prepared as the method described above. The TLC plates were activated by heating at 1200°C for about 30 min before use. Alcohol extract (2 µl) and Aqueous extract (2 µl) each were applied in duplicate, as tracks 1-6, with a band length of 8 mm each on a pre-coated silica gel 60 F254 TLC plate, with Linomat V applicator using a Hamilton syringe. No prewashing of the plate was done. Chamber saturation time was 18 h. The TLC plate was kept for development to a migration distance of 77 mm and scanned at 254 nm and 366 nm, band length 8 mm, slit dimension, scanning speed and source of radiation was Deuterium and Tungsten lamps respectively. The developed plates were dried and scanned successively at wavelengths of 254 nm, 366 nm and 425 nm, bandwidth, slit dimension, scanning speed and the source of radiation was deuterium, tungsten, and mercury. The Rf and peak area of the spots were interpreted using software.

b. Histochemical tests
   The roots sections were treated with various reagents showed the different colors (Table 1).
c. Powder evaluation
   Fragments of groups of cork cells, parenchyma cells, fragments of fiber bundle and fibers with narrow ends were observed. Fragments of secondary cortex with druses and rectangular type of calcium oxalate crystals along with the vessels with reticulate thickenings and bordered pits were observed. Powder treated with potassium iodide turns blue showing the presence of starch grains which are simple and oval (Figure 3).

**Physico-chemical constants**

Results of moisture content, ash and extractive values are presented. (Tables 2-4).

**Phyto-chemical analysis of B. tomentosa root**

Results of the study are shown. (Tables 5 and 6).
Fingerprinting Analysis of Alcohol and Aqueous Extracts

Alcohol and aqueous extract were developed in four different mobile phases for the detection of diverse phytoconstituents:

II. Ethyl Acetate: CH₃OH: C₂H₅OH: H₂O [8.1:1.1: 0.4:0.8] for glycosides

Alcohol extract (2 µl) of the root revealed the presence of 12 different phyto-constituents at Rf. 0.03, 0.09, 0.16, 0.21, 0.24, 0.28, 0.33, 0.40, 0.52, 0.57, 0.63, and 0.82 in mobile Phase I; 07 different phyto-constituents at Rf. 0.02, 0.05, 0.17, 0.21, 0.34, 0.46, and 0.60 in mobile Phase II; 7 different phyto-constituents at Rf. 0.27, 0.28, 0.40, 0.47, 0.56, 0.65, 0.72, 0.77, 0.83, and 0.96 in mobile Phase III; 12 different phyto-constituents at Rf. 0.01, 0.07, 0.18, 0.26, 0.28, 0.33, 0.42, 0.49, 0.63, 0.67, 0.69, 0.81 in mobile Phase IV respectively as shown in Figures 4-7 and Table 7.

The present study clearly indicates that the alcohol extract is rich in flavonoids, glycosides, phenolic compounds, and tannins. Alcohol extract showed prominent peak at Rf. 0.42, 0.49 and 0.55 quenched fluorescence at 254 nm and 366 nm, respectively.

Among these above peaks, peak corresponding to Rf. 0.42, 0.49 revealed the presence of 7-hydroxy flavones and marmesin, respectively.[15]

Aqueous extract (2 µl) of the root revealed 07 different phyto-constituents at Rf. 0.01, 0.03, 0.07, 0.09, 0.18, 0.32, 0.55, and 0.72 in mobile Phase I; 9 different phyto-constituents at Rf. 0.01, 0.04, 0.14, 0.22, 0.28, 0.31, 0.47, 0.59, and 0.72 in mobile Phase II; 07 phyto-constituents at Rf. 0.07, 0.26, 0.52, 0.65, 0.73, 0.86, 0.94 in mobile Phase III; 13 various phyto-constituents at Rf. 0.01, 0.07, 0.18, 0.26, 0.28, 0.33, 0.42, 0.49, 0.63, 0.67, 0.69, 0.81 in mobile Phase IV, respectively as shown in Table 8 and Figures 8-11.

The present study evidently showed that the aqueous extract is rich in glycosides and tannins. Aqueous extract showed a prominent peak at Rf. 0.22, 0.58, 0.70 and 0.94 quenched fluorescence at 254 nm and 366 nm, respectively.

Among these peaks, peak corresponding to Rf. 0.42, 0.49 revealed the presence of flavonoid 2.[16]

Anti-diabetic Activity Alcohol and Aqueous Extracts

OGTT of alcohol and aqueous extracts

Results of GTT of extracts B. tomentosa root alcohol extract (BTRAL) and B. tomentosa root aqueous extract (BTRAQ) were shown in Figure 13.
Anti-diabetic activity of BTRAL and BTRAQ

Results of anti-diabetic activity of extracts BTRAL and BTRAQ are shown in Figure 14.

DISCUSSION

Results of OGTT of BTRAL and BTRAQ extracts at doses (200, 400 mg/kg) have been showed. (Figure 13) The animal treated with aqueous extract at dose (200,400 mg/kg) of produced a statistically significant fall in fasting blood glucose level even after 1 h of extract administration, whereas alcohol extract failed to show the effect even after 2 h of extract administration.

Results of anti-hyperglycemic potential of BTRAL and BTRAQ extract have been showed above. BTRAL at low dose 200 mg/kg and BTRAQ extract at high dose 400 mg/kg showed statistically significant anti-diabetic activity and were found to be significant even after 14th and 21st day of treatment. Whereas high dose of BTRAL and low dose of BTRAQ were failed to showed the statistically significant anti-diabetic effect on rats. Different parameters such as body weight, food intake, water intake, weight of internal organs, were observed.

BTRAL (2 µl) revealed the 12 prominent peaks in ethyl Acetate: butanone: formic acid: water [5:3:1:1]; 11 prominent peaks in chloroform: methanol: water [6.5:2.5:0.4]; 10 prominent peaks in toluene: acetone: formic acid [4.5:4.5:1]; 12 prominent peaks in toluene: acetone [9:1], respectively.

BTRAQ (2 µl) revealed the 8 prominent peaks in ethyl Acetate: butanone: formic acid: water [5:3:1:1]; 11 prominent peaks in chloroform: methanol: water [6.5:2.5:0.4]; 10 prominent peaks in toluene: acetone: formic acid [4.5:4.5:1]; 12 prominent peaks in toluene: acetone [9:1].

8 prominent peaks were depicted in fingerprinting analysis of BTRAQ (2 µl) revealed the presence of in ethyl acetate: formic acid: acetic acid: water [10:1:1:1:2:6]; 9 prominent peaks in ethyl acetate: methanol: ethanol: water [8.1:1.1:0.4:0.8]; 7 prominent peaks in ethyl acetate: butanone: formic acids: water [5:3:1:1]; 10 prominent peaks in Iso-butanol: acetic acid: water [14:1:3.5], respectively. Among the above prominent peaks at Rf 0.60 belongs to the
Figure 4: High performance thin layer chromatography chromatogram of *Bauhinia tomentosa* root alcohol in mobile Phase-IV

Figure 5: High performance thin layer chromatography chromatogram of *Bauhinia tomentosa* root alcohol in mobile Phase-III

Figure 6: High performance thin layer chromatography chromatogram of *Bauhinia tomentosa* root alcohol in mobile Phase-II

Figure 7: High performance thin layer chromatography chromatogram of *Bauhinia tomentosa* root alcohol in mobile Phase-I
Figure 8: High performance thin layer chromatography chromatogram of *Bauhinia tomentosa* root aqueous in mobile Phase-IV

Figure 9: High performance thin layer chromatography chromatogram of *Bauhinia tomentosa* root aqueous in mobile Phase-III

Figure 10: High performance thin layer chromatography chromatogram of *Bauhinia tomentosa* root aqueous in mobile Phase-II

Figure 11: High performance thin layer chromatography chromatogram of *Bauhinia tomentosa* root aqueous in mobile Phase-I
Chrysin in the aqueous extract. BTRAQ was found to be rich in a range of flavonoids and phenolic compounds. BTRAQ extract showed prominent spot quenched fluorescence at 254 nm and 366 nm, respectively.

Pharmacognostical studies give the way of identification of plant as well as powder, which is the major problem while studying the herbal drugs. Microscopical studies showed the presence some important identifying characteristics such as multi-layered medullary rays, various types of crystals and starch grains along with the secondary xylem and phloem. Powder analysis is also important in the quality assurance studies showed the various elements along with fluorescence studies.

*B. tomentosa* root is one of the precious gift of nature to the human, is used under the Ayurvedic drug *Murva*. *Murva* is used in the treatment of various diseases including DM. The present study gives the scientific evidence and re-establish the *Murva* (*B. tomentosa*) possesses the statistically significant anti-diabetic activity as shown above in the results. Fingerprinting analysis of the extracts showed the presence of important phytoconstituents like Marmesin, chrysin, 7-hydroxy flavones, etc.[5]

CONCLUSION

The present study evaluates the anti-diabetic potential of *B. tomentosa* root along with fingerprinting and pharmacognostical analysis. The plant possesses the significant anti-diabetic activity and roots have shown the more significant activity than the stem of the plant as performed earlier. The presence of marmesin, chrysin, and 7-hydroxy flavones shows that extracts may be effective in the prevention of macro- and micro-vascular complications that are the major problem and challenge in the treatment of diabetes. Future prospects include the isolation and characterization of the phytoconstituents and ensuring the anti-diabetic activity at low dose.
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