EVALUATION OF IN VITRO ANTI-DIABETIC AND ANTI-INFLAMMATORY ACTIVITIES OF LEAVES EXTRACT OF BOEHMERIA RUGULOSA

ABHA SHUKLA, ANCHAL CHOUDHARY*
Department of Chemistry, Kanya Gurukula Campus, Gurukula Kangri Vishwavidyalaya, Haridwar, Uttarakhand, India.
Email: anchalkchoudhary@gmail.com
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ABSTRACT

Objective: The objective of the study is to evaluate in vitro antidiabetic and anti-inflammatory activity of different extracts of leaves of Boehmeria rugulosa by different methods.

Methods: In vitro α-glucose and α-amylase were used for antidiabetic activity and lipoxygenase, and protein denaturation method of inhibition assays was used to test anti-inflammatory activity. Successive extraction of leaves petroleum ether (PE), chloroform (CH), ethyl acetate (EA), acetone (AC), and ethanol (ETH) was performed, and extracts obtained from the extraction were applicable to these activities.

Results: The AC extract of leaves shows significantly in vitro antidiabetic activity, and AC has offered significant result 470.07±0.65 µg/mL in the inhibition of α-glucosidase and also for α-amylase assay 698.15±1.71 µg/mL. Acarbose was used as standard. In lipoxidase method, AC had shown better results and in protein denaturation method EA shown the higher inhibition (78.06±0.5 µg/ml) than the other extracts. The standard drug diclofenac sodium also offered significant inhibition against lipoxidase enzyme method with IC₅₀ value 21.76±1.29 µg/mL.

Conclusion: These findings suggest that the AC and EA possess potent antidiabetic and anti-inflammatory activities in vitro conditions.

Keywords: Anti-inflammatory, Antidiabetic, Boehmeria rugulosa, Diclofenac sodium, Acarbose.

INTRODUCTION

Boehmeria rugulosa belongs to the family Urticaceae, and it is found in tropical and subtropical region of Himalayan. The plant is widely used in traditional system of medicine for human health. Leaves extracts obtained with different solvents were contained polyphenols, tannins, saponins, carbohydrates, fatty acids, and various trace elements which are responsible for the various biological activities. Some isolated and identified phytochemicals from leaves have been demonstrated to own significant biological properties [1]. Diabetes mellitus (DM) is characterized by a group of chronic endocrine disorder in which the deficiency of insulin causes glucose to accumulate in the blood, leading to a group of metabolic diseases [2]. Currently, there is growing interest in herbal remedies due to the lesser side, low cost, and better response for the treatment of DM [3]. More than 400 plants worldwide have been documented as beneficial in the treatment of diabetes [4,5].

The mechanism of the anti-inflammatory activity are shown by two different pathway. In the present study, plant extract shows the inhibition by protein denaturation method [6,7]. The mechanisms of inflammation which involve the metabolism of anachronic acid play an important role. It can be metabolized by the 5-lipoxygenase (5-LOX) pathway to hydroperoxyeicosatetraenoic acids (HPETEs) and leukotrienes (LTs), which are important biologically active mediators in a variety of inflammatory events. In 5-LOX pathways, appropriate stimulation of neutrophils, anachronic acid is cleaved from membrane phospholipids and can be converted to LTs. Inhibition of 5-LOX leads to decreasing production of LTs. Furthermore, inflammatory processes also involve reactive oxygen species started by leukocyte activation. Therefore, antioxidant properties may provide important information about the potential activity of a drug on inflammatory processes [8].

The lipoxygenase pathways of arachidonic acid catabolism designed above. Algogenic compounds are shown in bold type and enzymes which are shown in italics. HETE: Hydroxyeicosatetraenoic acids, HPETE: Hydroperoxyeicosatetraenoic acids, LT: Leukotriene.

A survey of literature indicated no research work has been conducted to evaluate the anti-inflammatory and antidiabetic potential of B. rugulosa leaves by in vitro method. The present study concerns the determination of anti-inflammatory activity of by inhibition of albumin denaturation and anti-lipoxygenase activity, however, antidiabetic activity by α-amylase and α-glycosidase methods.
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METHODS

Standards and reagents

Bovine serum albumin, lipoxidase enzyme, and linoleic acid were obtained from Sigma-Aldrich, Tris buffer (Merck), p-Nitrophenyl-α-D-glucopyranoside (SRL Pvt., Ltd.), α-amylase (SRL Pvt., Ltd.), dimethyl superoxide (DMSO) (Merck), 3,5-dinitrosalicylic acid (DNSA) (SRL Pvt., Ltd.), α-glucosidase (SRL Pvt Ltd); and acarbose (Bayer India Limited), sodium carbonate (CDH), petroleum ether (PE) (Merck), ethanol (ETH) (Merck), ethyl acetate (EA) (Merck), and acetone (AC) (Merck) were purchased. All other solvents and chemicals used were analytical grade.

Collection of plant material

The bark of plant B. rugulosa was collected from the Rishikesh region. Voucher specimens have been put in the Herbarium of the Botanical Survey of India, Dehradun, in November 2015, with accession no. 115901. A voucher specimen has been deposited in medicinal plants Herbarium Department of Chemistry, Kanya Gurukula Campus, Gurukula Kangri Vishwavidyalaya, to registry no. 1/4. The plant materials were washed, dried in shade and ground to powder, and stored in polythene bags for further use.

Preparation of extracts and phytochemical screening

Plant extraction was done by the Soxhlet extraction process with different solvents. Phytochemical screening was carried out by standards of analytic methods [9].

Antidiabetic activity

In vitro methods employed in antidiabetic activity of each extract by α-amylase and α-glucosidase inhibitory assay.

α-amylase inhibition activity

The α-amylase inhibitory activity of extracts was performed using DNSA method with a slight modification [10,11].

A total of 1 ml (1–1000 μg/ml) of test samples and standard drug (100–1000 μg/ml) were added to 1 ml of 20 mM phosphate buffer (pH 6.9) containing α-amylase (3 mg/ml) solution and were incubated at 37°C for 30 min. After these, 1 ml of 1% starch solution in 20 mM phosphate buffers (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 37°C for 15 min. The reaction was stopped with 1 ml of DNSA color reagent. The test tubes were then incubated in a boiling water bath for 5 min, and cooled to room temperature. The reaction mixture was then diluted after adding 9 ml distilled water, and absorbance was measured at 540 nm.

For correcting background absorbance (absorbance due to extracts or standard), the enzyme was substituted for 1 ml buffer solution with similar test procedure. The α-amylase inhibitory activity was obtained by the equation.

\[
\text{α-amylase inhibitory activity (}%\text{inhibition)} = \left(\frac{A_{C} - A_{C}}{A_{S} - A_{B}}\right) \times 100
\]

Where AC represents absorbance of pure control having 100% enzyme activity (DNSO and enzyme), AC symbolizes absorbance of blank for pure control having 0% enzyme activity (DNSO and buffer), AS represents absorbance of sample or standard (sample/standard and enzyme), and AB symbolizes background absorbance due to sample and standard (sample/standard and buffer). IC_{50} of each extract and standard acarbose was calculated by the graphical method by plotting % inhibition versus concentration.

α-glucosidase inhibition activity

The inhibitory activity was determined in accordance with according to Andrade-Getto et al. [12] with a minor modification [13]. Briefly, 1 ml of each solution of different concentrations (1–5000 μg/ml) of extracts or standard acarbose in DMSO was incubated with 1 ml of α-glucosidase (1 U/ml in 100 mM phosphate buffer pH 6.8) for 30 min at 37°C. The enzyme reaction is started by adding 1 ml of p-nitrophenyl-α-glucopyranoside in 100 mM phosphate buffer (pH 6.8). The reaction mixtures were then incubated for 15 min at 37°C. The reaction was stopped by adding 4 ml 0.5 M Tris buffer. The absorbance was taken by UV-VIS spectrophotometer (Agilent Technologies Cary-60) at 410 nm.

For correcting background absorbance, the enzyme was replaced by 1 ml buffer solution with similar test procedure. The % inhibition and IC_{50} were calculated in a similar way as mentioned in α-amylase activity. Earlier 0.1 M NaOH was used to stop the reaction. Each test was performed three times, and the mean absorption was used to calculate the percentage α-glucosidase inhibition. The control samples were also prepared accordingly without any plant extracts and were compared with the test samples containing the plant extracts prepared with different solvents.

\[
\text{α-glucosidase inhibitory activity (}%\text{inhibition)} = \left(\frac{A_{C} - A_{C}}{A_{S} - A_{B}}\right) \times 100
\]

Anti-inflammatory activity

Inhibition of albumin denaturation

The anti-inflammatory activity of a plant extract was studied using inhibition of albumin denaturation technique which was studied according to Mizushima and Kobayashi [14] and Sakat et al. [15] followed with minor modifications. The reaction mixture (0.5 ml; pH 6.3) consisted of 0.45 ml of bovine serum albumin (5% aqueous solution) and 0.05 ml of distilled water. pH was adjusted at 6.3 using a small amount of 1 N HCl. Different concentrations of plant extract (50–1000 μg/ml) were added to the reaction mixture and were incubated at 37°C for 20 min and then heated at 60°C for 10 min, and after cooling the samples, 2.5 ml of phosphate buffer saline was added. Turbidity was measured spectrophotometrically at 660 nm. The percentage inhibition of protein denaturation was calculated as follows:

\[
\text{Percentage inhibition (}%\text{)} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100
\]

Anti-lipoxygenase assay

Anti-lipoxygenase assay was studied using linoleic acid as substrate and lipoxidase as an enzyme. The test solution was dissolved in 0.25 ml of 2M borate buffer pH 9.0 and added 0.25 ml of linoleic acid solution (0.6 mM) and 0.05 ml of distilled water; pH was adjusted at 6.3 using a small amount of 1 N HCl. Different concentrations of plant extract (50–1000 μg/ml) were added to the reaction mixture and were incubated at 37°C for 20 min and then heated at 60°C for 10 min, and after cooling the samples, 2.5 ml of phosphate buffer saline was added. Turbidity was measured spectrophotometrically at 660 nm. The percentage inhibition of lipoxygenase assay was calculated using the following formula:

\[
\text{Percent inhibition (}%\text{)} = \left(\frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}}\right) \times 100
\]

Where A_{control} is the absorbance of the control reaction and A_{test} is the absorbance of the control reaction to extract.

RESULTS AND DISCUSSION

Antidiabetic activity

α-Amylase inhibition activity

The in vitro α-amylase inhibitory activity of B. rugulosa leaves extracts compared with acarbose is illustrated in Table 1. All the extracts showed by signifying results. Acarbose showed percentage α-amylase inhibition in varying concentration from (1–1000 μg/ml) with an IC_{50} value corresponding to greater potential and better therapeutic efficacy. AC extract is taken into consideration in the highest α-amylase inhibitory activity. It can be viewed as an excellent inhibitory activity.
α-glucosidase inhibitory activity

The *in vitro* α-glucosidase inhibitory activity of *B. rugulosa* leaves extracts compared with acarbose is illustrated in Table 2.

The α-glucosidase inhibition on changing the concentration of each extract helps in an estimation of IC₅₀ value of each extract as well as standard acarbose [17]. All the extracts showed inhibitory effects toward α-glucosidase. The AC extract had the highest inhibition activity (470.07 ± 0.65 µg/ml) while ETH had the lowest inhibition (1152.27 ± 0.37 µg/ml).

In the present study, leaves extracts were evaluated for antidiabetic activity and acarbose was used as a standard reference. The finding of the results reveals that the AC shows good results followed by the EA.

Anti-inflammatory activity

*Inhibition of albumin denaturation*

Protein denaturation is a process whereby proteins lose their tertiary structure and secondary structure by application of external stress, such as strong acid or base, a concentrated inorganic salt, and an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation of the mechanism of the anti-inflammatory activity, ability of plant extract to inhibit protein denaturation was examined [18]. It had successfully inhibiting heat-induced albumin denaturation. Maximum inhibition of 78.06% was measured at 500 µg/ml. Diclofenac, a standard anti-inflammation drug, showed the maximum inhibition 94.20% at the concentration 500 µg/ml in Table 3.

Comparison of all extract inhibition with the standard is shown in the Fig. 1 in and their % inhibition is shown in Table 3 for comparison purpose. The EA extract of leaves indicated the highest inhibition compared to other extracts from inhibition 78.06 µg/ml.

**Anti-lipoxygenase activity**

The establishment of new *in vitro* test systems has stimulated the screening of plants aiming to find the development of new drugs [19]. IC₅₀ values are given in the Table 4.

It is expected to result in comparison with the standard diclofenac sodium with IC₅₀ value 21.76 µg/ml. AC extract shows better results than the other extracts, which shows their potential in inflammation. The 5-LOX leads to the formation of biologically active lipoxins. Whereas, it is leading the formation of 5, 6-epoxy LTs, which are responsible for inflammation. In the present study, leaves extracts show the significant consequences for *in vitro* anti-inflammatory activity. In protein denaturation assay, EA extract shows better results which were followed by AC. Now, EA extract shows comparable inhibition with reference standards followed by AC.

**CONCLUSION**

In the present study, leaves extracts indicate the significant anti-diabetic and anti-inflammatory activities by *in vitro* α-glucosidase and α-amylase assay for anti-diabetic and heat-induced protein denaturation, and 5-LOX enzyme methods apply for anti-inflammatory activity. Our results showed that AC extract of leaves was better for anti-diabetic and anti-inflammatory activities followed by the EA extracts by *in vitro* conditions. These findings suggest that AC and EA were more potent for these activities. However, further studies currently undergo a process for different parts of this plant for biological activities.

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**AUTHORS’ CONTRIBUTION**

Anchal - conceptualization of paper and compilation full manuscript.

Abha Shukla - Reviewing and checking of the manuscript in improving the level of the manuscript.

**CONFLICTS OF INTEREST**

There are no conflicts of interest.

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**Table 1: IC₅₀ value of leaves extracts for α-amylase inhibition assay**

| Extracts/Standards | IC₅₀ Value  |
|--------------------|------------|
| Acrbose            | 617.23±0.15 |
| Petroleum ether    | ND         |
| Chloroform         | 1310.21±0.46 |
| Ethyl acetate      | 820.34±1.12 |
| Acetone            | 698.15±1.71 |
| Ethanol            | 497.68±1.12 |

ND: Not detectable

**Table 2: IC₅₀ value of leaves extract for α-glucosidase inhibition assay**

| Extracts/Standards | IC₅₀ Value  |
|--------------------|------------|
| Acrbose            | 358.42±1.52 |
| Petroleum ether    | ND         |
| Chloroform         | 875.89±0.93 |
| Ethyl acetate      | 624.84±0.78 |
| Acetone            | 470.07±0.65 |
| Ethanol            | 1152.27±0.37 |

ND: Not detectable

**Table 3: Effect of leaves extracts on heat-induced protein denaturation treatment concentration (500 µg/ml) and absorbance at 660 nm**

| Extracts/standards | Concentration (µg/ml) | % Inhibition |
|--------------------|-----------------------|--------------|
| Diclofenac sodium  | 500                   | 94.20±0.65   |
| Petroleum ether    | 500                   | 63.20±1.12   |
| Chloroform         | 500                   | 64.47±1.36   |
| Ethyl acetate      | 500                   | 78.06±0.5    |
| Acetone            | 500                   | 71.81±1.72   |
| Ethanol            | 500                   | 34.50±1.52   |

**Table 4: IC₅₀ value of leaves extracts with standards for lipoxygenase inhibition assay**

| Extracts/standards | IC₅₀ values  |
|--------------------|--------------|
| Diclofenac sodium  | 21.76±1.29   |
| Petroleum ether    | ND           |
| Chloroform         | 254.46±0.7   |
| Ethyl acetate      | 249.89±5.97  |
| Acetone            | 89.07±0.85   |
| Ethanol            | 151.27±1.2   |

ND: Not detectable
REFERENCES

1. Semwal DK, Rawat U, Semwal R, Singh R, Krishan P, Singh GJ. Chemical constituents from the leaves of *Boehmeria rugulosa* with antidiabetic and antimicrobial activities. J Asian Nat Prod Res 2009;11:1045-55.

2. Das P, Devi PV, Yasmine Y. Assessment of *in vitro* anti-diabetic activity of *Ficus glomerata* Merrina. Pharm Lett 2016;8:267-72.

3. Day C, Bailey CJ. Hypoglycemic agents from traditional plant treatments for diabetes. Int Ind Biotech 1998;50:5-8.

4. Gray AM, Flatt PR. Nature’s own pharmacy: The diabetes perspective. Proc Nutr Soc 1997;56:507-17.

5. Swanston-Flatt SK, Flatt PR, Day C, Bailey CJ. Traditional dietary adjuncts for the treatment of diabetes mellitus. Proc Nutr Soc 1991;50:641-50.

6. Leelaprakash G, Dass SM. *In-vitro* anti-inflammatory activity of methanol extract of *Enicostemma axillare*. Int J Drug Dev Res 2010;3:189-96.

7. Ingle PV, Patel DM. C-reactive protein in various disease condition - an overview. Asian J Pharm Clin Res 2011;4:9-13.

8. Anoop MV, Bindu AR. *In-vitro* anti-inflammatory activity studies on *Syzygium zeylanicum* (L.) DC Leaves. Int J Pharm Res Rev 2015;4:18-27.

9. Anchal C, Shukla A, Shukla RK, Tyagi R. Evaluation of Antioxidant and antidiabetic capacity of plant *Boehmeria rugulosa* bark. Int J Chemtech Res 2017;10:324-32.

10. Hansawadi C, Kawabata J, Kasai T. Alpha amylase inhibitors from Roselle (*Hibiscus sabdariffa*). Biosci Biotechnol Biochem 2000;64:1041-3.

11. Rengasamy S, Thangaprakasam U. Isolation, screening and determination of α-amylase activity from *Marine streptomyces* species. Int J Pharm Sci 2018;10:122-7.

12. Andrade-Cetto A, Becerra-Jimenez J, Cardenas-Vazquez R. Alpha-glucosidase inhibiting activity of some Mexican plants used in the treatment of Type 2 diabetes. J Ethnopharmacol 2008;116:27-32.

13. Li D, Ni JM. Preliminary study of an alpha-glucosidase inhibition from the roots and stems of *polygonatum sibiricum* Red. Asian J Tradit Med 2008;3:179-85.

14. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. J Pharm Pharmacol 1968;20:169-73.

15. Sakat S, Juvekar AR, Gumbhire MN. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int J Pharm Pharm Sci 2010;2:146-55.

16. Cashman JN. The mechanisms of action of NSAIDs in analgesia. Drugs 1996;52:13-23.

17. Singh N, Gupta AK, Juyal V, Deepak P, Gahlot M. Phytochemical screening and blood glucose level lowering effect of *Bergenia ligulata* root extracts. J Nat Remedies 2011;11:19-23.

18. Reddenna P, Whelan J, Maddipati KR, Reddy CC. Purification of arachidonate 5-LOX from potato tubers. Methods Enzymol 1990;187:268-77.

19. Shinde UA, Kulkarni KR, Phadke AS, Nair AM, Dikshit VJ, Mungantiwar AA, et al. Mast cell stabilizing and lipoygenase inhibitory activity of *Cedrus deodara* Roxb loud wood oil. Indian J Exp Biol 1999;37:258-61.