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Research Article

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

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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 measure 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 IC_{50} 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.

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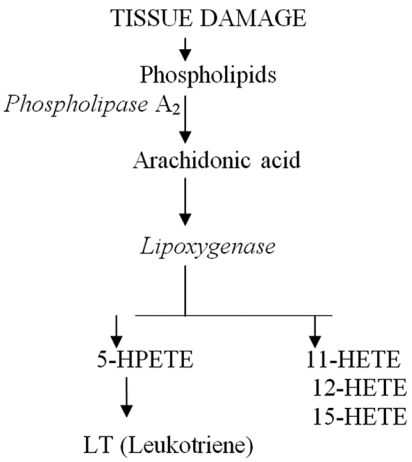
INTRODUCTION

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.

METHODS

Standards and reagents

Brovine 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 chemical 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-glycosidase 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 a 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.

$$\alpha\text{-amylase inhibitory activity } (\%\text{inhibition}) = \frac{\left[\left(AC^{+} - AC^{-}\right) - \left(AS - AB\right)\right]}{\left(AC^{+} - AC^{-}\right)} \times 100$$

Where AC+ represents absorbance of pure control having 100% enzyme activity (DMSO and enzyme), AC− symbolizes absorbance of blank for pure control having 0% enzyme activity (DMSO and buffer), AS represents absorbance of sample or standard (sample/standard and enzyme), and AB symbolizes for 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-Cetto *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-dglucopyranoside 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 proceduree. The % inhibition and IC_{50} were calculated in a similar way as mentioned in α-amylase activityy. 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.

$$\alpha\text{-glucosidase inhibitory activity } (\%\text{Inhibition}) = \frac{\left[\left(AC^{+} - AC^{-}\right) - \left(AS - AB\right)\right]}{\left(AC^{+} - AC^{-}\right)} \times 100$$

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:

$$\text{Percentage inhibition } (\%) = \frac{\left(Abs_{Control} - Abs_{sample}\right)}{Abs_{Control}} \times 100$$

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 lipoxidase enzyme solution (20,000 U/ml) and incubated for 5 min. at 25°C. After which, 1 ml of linoleic acid solution (0.6 mM) was added, mixed well, and absorbance was measured at 234 nm. Diclofenac was used as the reference standard [16]. Percentage inhibition of lipoxygenase assay was calculated using the following formula:

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

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 had shown signifying results. Acarbose showed percentage alpha-amylase inhibition of in varying concentration from (1–1000 µg/ml) with an IC_{50}. Lower IC_{50} value corresponds to greater potential and better therapeutic efficacy. AC extract is taken into consideration in the highest alpha-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 IC_{50} 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 78.061% 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.

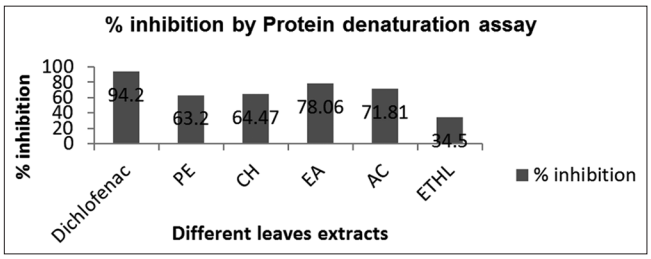


Fig. 1: Comparision of leaves extract with standards

Table 1: IC_{50} value of leaves extracts for α-amylase inhibition assay

Extracts/Standards	IC_{50} value
Acarbose	617.23±0.15
Petroleum ether	ND
Chloroform	1310.21±0.46
Ethyl acetate	820.34±1.3
Acetone	698.15±1.71
Ethanol	497.68±1.12

ND: Not detectable

Table 2: IC_{50} value of leaves extract for α-glucosidase inhibition assay

Extracts/Standards	IC_{50} value
Acarbose	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

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]. IC_{50} values are given in the Table 4.

It is expected to result to comparison with the standard diclofenac sodium with IC_{50} 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 antidiabetic and anti-inflammatory activities by *in vitro* α-glucosidase and alpha-amylase assay for antidiabetic and heat-induced protein denaturation, and 5-LOX enzyme methods apply for anti-inflammatory activity. Our results showed that AC extract of leaves was batter for antidiabetic 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.

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±1.11
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: IC_{50} value of leaves extracts with standards for lipoxidase inhibition assay

Extracts/standards	IC_{50} 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

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