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Phytochemical Screening and Biological Activities of *Impatiens balsamina.* L seeds

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Abstract

Background and Objective : In the present study, seeds of *Impatiens balsamina* L are subjected to phytochemical evaluation and biological activities like, antioxidant, antidiabetic and antiinflamatory studies *in-vitro*. ***Methodology***: Antioxidant activity is studied using reducing power assay and phospho molybdenum assay, the results were compared with standard ascorbic acid. Free radical scavenging assay % of inhibition is calculated. Antidiabetic and Anti inflammatory activity were calculated by Amylase inhibition and BSA denaturation assay respectively. ***Results:*** Phytochemical screening of the ethanol extract of seeds revealed the presence of alkaloids, flavanoids, terpenoids, tannins and saponins. IC_{50} value of extract is found to be 320µg/ml. , Seed extract exhibited significant anti-diabetic activity as compared to standard drug in-vitro. For invitro studies for anti-inflammatory assay, IC_{50} value was found to be 210 µg/ml. The study demonstrates significant analgesic and anti-inflammatory effects of aqueous extract of Impatiens balsamina leaves.

Keywords: *Impatiens balsamina L.,* anti-inflammatory, anti diabetic, IC_{50}.

1. Introduction

In India, drugs of herbal origin have been used in traditional systems of medicines such as *Unani* and *Ayurveda* since ancient times[1]. The drugs are derived either from the whole plant or from different organs, like leaves, stem, bark, root, flower, seed, etc. Some drugs are prepared from excretory plant product such as gum, resins and latex. In many of the developing countries the use of plant drugs is increasing because modern life saving drugs is beyond the reach of three quarters of the third world's population. With the rapid depletion of forests, impairing the availability of raw drugs, Ayurveda, like other systems of herbal medicines has reached a very critical phase. About 50% of the tropical forests, the treasure house of plant and animal diversity have already been destroyed[2]. Green plants synthesize and preserve a variety of biochemical products, many of which are extractable and used as chemical feed stocks or as raw material for various scientific investigations. Many secondary metabolites of plant are commercially important and find use in a number of pharmaceutical compounds. In some cases, the crude extract of medicinal plants may be used as medicaments. On the other hand, the isolation and identification of the active principles and elucidation of the mechanism of action of a drug is of paramount importance. The scientific study of traditional medicines, derivation of drugs through bioprospecting and systematic conservation of the concerned medicinal plants are thus of great importance.

Impatiens balsamina L is an annual plant belongs to family *Balsaminaceae* commonly called as Balsam, Jewelweed. The average height of the plant is about 1 to 2.5 feet. It is a very variable species particularly in the size of leaves and flower these plants tend to grow in wet shady soils along stream banks. Leaves are of 2 to 4 inches, simple, pinnate venation, lanceolate shaped, serrate margin and green in colour. Flowers are of different colours like white; pink; salmon; purple; lavender. The genus has been widely distributed throughout the northern hemisphere and native of India and parts of mainland South East Asia. (**Figure I**)

Inflammation is a complex reaction to injurious agents, such as microbes and damaged usually necrotic, cells that consist of vascular responses, migration and activation of leucocytes, and systemic reactions. In BSA assay thermal immunogenic bovine serum albumin (BSA) by natural products is a feature that could be developed for selecting therapeutically interesting molecules without the use of animals.

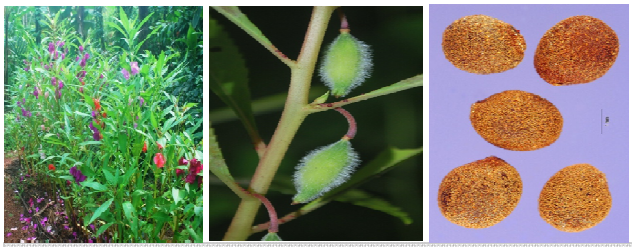

Figure I: The experimental plant with its seeds

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The above assay was developed from research done by Grant *et al*.

Diabetes is a defect in the body's ability to convert glucose (sugar) to energy. Carbohydrates, when digested, change to glucose. In order for glucose to be transferred from the blood into the cells, the hormone - insulin is needed. Insulin is produced by the beta cells in the pancreas (the organ that produces insulin).In individuals with diabetes, this process is impaired. Diabetes develops when the pancreas fails to produce sufficient quantities of insulin Type 1 diabetes or the insulin produced is defective and cannot move glucose into the cells. In Type 2 diabetes. Either insulin is not produced in sufficient quantities or the insulin produced is defective and cannot move the glucose into the cells. *In vitro* screening of anti-diabetic drug is carried out by estimating the levels of PPARγ and the two principle enzymes which involve in the carbohydrate digestion and glucose absorption process. In the current study we choosen inhibition of α-Amylase assay. Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitors bind to alpha-bond of polysaccharide and prevent break down of polysaccharide in mono and disaccharide. Due to suppression of the activity of enzymes amylase would delay the degradation of starch and oligosaccharides, which would in turn cause a decrease in the absorption of glucose.

In Korea, *Impatiens balsamina* has been used in traditional oriental medicine to treat scrofulosis, carbuncles, and dysentery[6] . Kaempferol widely present in *Impatiens balsamina,* was a markedly active inhibitor of transcriptional activation of COX-2[7]. Kaempferol and quercetin have inhibitory activities against melanin synthesis[8]. Juice from the white corolla is painted topically on the skin as an anti-pruritic to treat several types of dermatitis and multiple antibiotic-resistant against Helicobacter pylori[10]. Flowers are useful when applied to burns and scalds. It acts as an emetic, cathartic and diuretic. It is found abundantly in Manipur and Assam, INDIA and is widely used for its medicinal properties[11,12]. Even though there are various reports of the uses of *Impatiens balsamina* there is lack of scientific data to substantiate them. Therefore, In the present study, we have evaluated invitro antidiabetic activity, antioxidant and anti inflammatory activity of indian species of I*mpatiens balsamina*.

2. Methodology

2.1 Collection of Sample:

The seeds of the plant *Impatiens balsamina.*L were collected from Nursery of Lalbhag Botanical garden, Bangalore, and Authenticated by Botanist.

2.2 Chemicals and Reagents:

All the chemicals and reagents used were of analytical grade and are purchased from Lancaster Research Lab, Chennai, India and Himedia Lab, Mumbai, India.

2.3 Preparation of Extract:

Ethanolic extract: The seeds of the plant collected were dried under low sunlight for 7-8 days and then grinded to a fine powders in a grinder. The powdered plant material (15g) was subjected to maceration using ethanol for 4 days, then filtered with muslin cloth and evaporated to dryness. Extract was kept in desiccator.

2.4 Phytochemical analysis:

The seed extract was used for preliminary screening of phytochemicals such as alkaloids (Wagner's and Meyer's tests), saponins (foam test), tannins (gelatin test), and flavonoids (Alkaline reagent and Lead acetate tests), the screening was done as per the standard method.

2.4.1 Test for alkaloids[13]:

2.4.1.1 Dragendroff's test: 2 mg of the test extract. Add 5ml of distilled water & 2M HCl was added untill an acidic reaction will occur. To this 1ml Dragendroff's reagent was added. The Formation of orange red precipitate indicates the presence of alkaloids.

2.4.1.2 Hager's test: 2mg of the extract in a test tube. To this add Few drops of Hager's reagent. Formation of yellow precipitate confirms the presence of alkaloids.

2.4.1.3 Wagner's test: Take 2mg of the test extract. Acidified it with 1.5% v/v of HCl and add few drops of Wagner's reagent. Formation of brown/reddish brown precipitate indicates the presence of alkaloids.

2.4.1.4 Mayer's test: 2mg of extract. Add few drops of Mayers reagent. Formation of white/yellow precipitation indicates the presence of alkaloids.

2.4.2 Test for flavanoids[14]:

Ferric chloride test: Test solution adds few drops of ferric chloride solution. Intense green colour indicates the presence of flavanoids.

Zinc hydrochloride and reduction test: Test solution. Add zinc dust and few ml of HCl. Presence of red colour indicate the presence of flavanoids.

Lead acetate solution test: Test solution.Add few drops of (10%) lead acetate Presence of yellow precipitate shows the presence of flavanoids.

2.4.3 Test for terpenoids[15]: Weigh about 0.5 gm of plant extract in a separate test tube with 2ml of chloroform. Add concentrated sulphuric acid carefully to form a layer. Observe for the presence of reddish brown colour interface to show positive result for the presence of terpenoids.

2.4.4 Test for saponins[15]: To the extract solution a drop of sodium bicarbonate solution was added. The mixture was shaken vigourously and left for 3 minutes. The formation of honey comb like froth indicates the presence of saponins.

2.4.5 Test for tanins[15]: 1-2ml of extract and add few drops of 5% w/v ferric chloride. Green clour indicates the presence of gallotanins. White brown colour indicates the presence of pseudotanins.

2.4.2.6 Gelatin test: 1-2ml of extract, Add few drops of gelatin test. White precipitate indicates the presence of gelatin.

2.5 Antioxidant activity

2.5.1 Reducing power assay[16]

Plant extract of different concentration ranging from 100µg-500µg (0.1-0.5) was taken in six test tubes. Make up the volume to 1ml with ethanol. 1ml ethanol is taken as blank. The extracts are now mixed with 2.5 ml (0.2M, pH 6.6) phosphate buffer and

2.5ml (0.1%) potassium ferricyanide. This was incubated at 50^0 C for 20 minutes. Then 2.5ml of TCA is added to all the test tubes and extracts are centrifuge at 3000 rpm for 20 minutes. The supernatant was taken 2.5ml in each tube and was mixed with 2.5ml of distilled water and 0.5ml 0.1% ferric chloride. The solution absorbance is measured at 700nM. Increasing absorbance of the reaction mixture indicates increasing reducing power.

2.5.2 Phospho molybdenum assay[17]:

Plant extract of different concentration ranging from100-500µg (0.1-0.5ml) and the volume is made up to 1ml with ethanol. 1ml of Distilled water is taken as blank. The extract was mixed with1ml each of reagent solutions (0.6M H_2SO_4, 28mM NaH_2PO_4 (Sodium Phosphate) & 4mM ammonium molybdate). The reaction mixture was incubated at 95^0C for 90 minutes and cooled to room temperature. Finally, absorbance was measured at 695nM using spectrophotometer against blank.

2.5.3 DPPH free radical scavenging assay:

Modified Goyal *et al* (2010) protocol was used for DPPH scavenging activity[18]. Plant extract of different concentration ranging from 100-500µg (0.1 ml-0.5ml) was taken in test tubes and make the volume to 1 ml with methanol. Add 4ml of DPPH solution. Measure the Optical density at 517 nm against Blank containing 5 ml of methanol, and control is containing 1 ml methanol and 4 ml of DPPH. The % of inhibition is calculated by using formula

$$\% \text{ of Inhibition} = \frac{AbC - AbS}{AbC} \times 100$$

Where, AbC is absorbance of control
AbS is absorbance of sample.

2.6 *In-vitro* Antidiabetic activity[19]

2.6.1 Inhibition of alpha amylase enzyme:

Standard maltose curve: 0.2-1ml of standard maltose (1mg/ml) taken into different test tubes. Make the volume to 1.0 ml in each case with distilled water. Added 1.0ml of DNS reagent to the each tube & then placed all the tubes in boiling water bath for 15 minutes. Added 8.0 ml of distilled water in each test tube & Mix the contents of the tube thoroughly. Then read the absorbance of the solution in calorimeter at 570 nM against blank solution.

Alpha Amylase inhibition assay:

100-500µl (100-500µg) of extract was taken into different test tubes. Made the volume to 0.5ml with phosphate buffer of pH 6.9, Blank was measured by taking 1 ml of phosphate buffer. Control was measured by taking 0.5ml of phosphate buffer. The solution was then treated with 0.5ml of alpha amylase (0.5mg/ml). The solution was incubated at 25^oC for 10 minutes. Added 0.5ml of 1% starch solution in 0.02 M sodium phosphate buffer of pH 6.9 to all the tubes, and then incubate at 25^oC for 10 minutes. The reaction was stopped by adding 1.0 ml of DNS and the reaction mixture was kept in boiling water bath for 5 minutes, cooled to room temperature. The solution was mixed with 8 ml distilled water. Read the absorbance of the solution in calorimeter at 570 nM against blank solution.

Amount of maltose produced is calculated using standard maltose curve, and Enzyme activity is calculated by using formula

$$\text{Enzyme activity} = \frac{\text{Amount of maltose formed} \times 2}{10 \times 342}$$

2.7 *In-vitro* Anti inflammatory activity[20]

2.7.1 BSA Denaturation assay:

100-500µl (100-500µg) was taken in different test tubes. The volume was made up to 1 ml with methanol. 1ml of BSA was added in each tube & incubated at Room temperature for 10 minutes. The tubes were then kept in water bath at 60^0 C for 5-10 minutes. The optical density was measured at 660nM against blank containg 1 ml of distilled water and control containing 1 ml of methanol and standard drug sodium diclofenac (10mg/10 ml).

$$\% \text{ of Inhibition} = \frac{AbC - AbS}{AbC} \times 100$$

Where, AbC is absorbance of control, AbS is absorbance of sample.

3. Results and Discussion

3.1 Phytochemical analysis

Preliminary phytochemical screening of ethanolic extract of the dried seeds revealed the presence of secondary metabolites like alkaloids, tannins, and flavonoids. The results are tabulated in **Table I**.

Table I: Preliminary Phytochemical detection

Test for alkaloids	Results
Dragendroff"s test:	++
Hager"s test:	++
Wagner"s test:	++
Mayer"s test:	++
Test for flavanoids;	
Ferric chloride test:	++
Zinc test:	++
Lead acetate solution test:	++
Test for terpenoids:	++
Test for saponins:	
Foam test:	++
Test for tanins:	++
Gelatin test:	++

Plant extract exhibited high reducing power activity. Highest reducing power activity was seen at 500 µg/ml. Increasing activity indicates dose dependent properties of the extract. As the dose increases , antioxidant activity also increases. (**Table II**) DPPH assay is a result of conversion of DPPH radical to DPPH stable molecule by extraction of H atom from antioxidant, DPPH radical absorb light higher than the stable DPPH, therefore when mixed with antioxidant rich plant extract the absorbency decreased at 517nm . A dose response curve was plotted to determine the IC50 values. IC50 is defined as the concentration

Table II: Reducing Power and Phospho molybdenum assay of plant extract

Extract (µg/ml)	% of Anioxidant activity (Reducing Power assay)	% of Anioxidant activity (Phospho molybdenum assay)
100	7.29	53.8
200	16.37	59.6
300	25.90	68.91
400	42.77	70.96
500	74.33	71.43

sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged. The standards used in this assay were vitamin C (Sigma, USA). The results indicate that the extract has better DPPH scavenging action and reducing power compared to the standard used in the study. (**Figure II**)

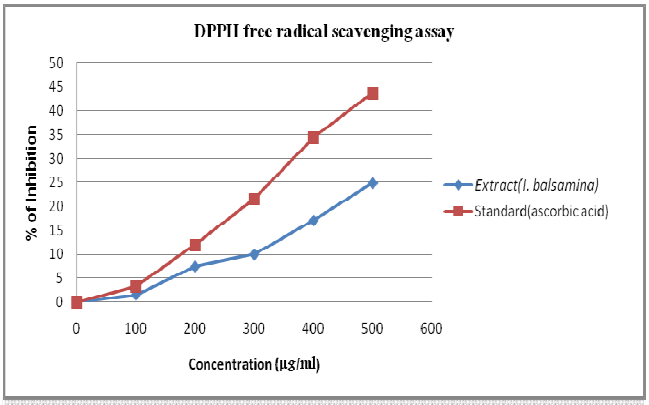

Figure II: DPPH scavenging assay

Our results are in accordance with several previous findings[12]. From research investigations, it was observed that the increase in reducing activity depends on the percentage of antioxidant activity[18]. Maximum activity was seen in 500µg/ml concentration. IC_{50} 320 µg/ml was found in DPPH free radical scavenging activity compared to the standard Ascorbic acid with

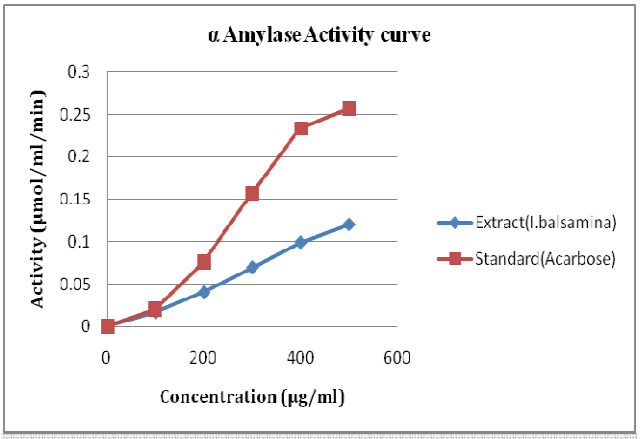

Figure II: *Invitro* antidiabetic activity of extract

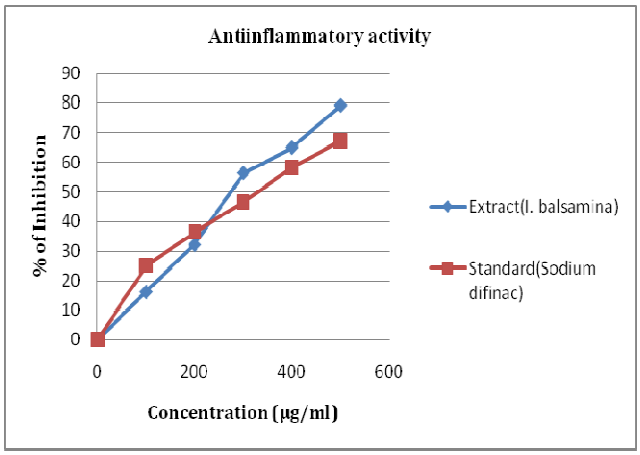

Figure IV: Anti-inflammatory activity of plant extract using BSA denaturation activity

IC_{50} 310µg/ml. Extract was also shown very good antiinflammatory with IC_{50} value of Standard and extract is found to be 196 µg/ml and 210 µg/ml respectively. This might be because of Cyclooxygenase-2 Inhibition by 1, 4-Naphthoquinones present in *Impatiens balsamina* L.[21]. Finally results are infirmed that extract was also shown invitro andidiabetic activity. (**Figure III & IV**). Lim et al had shown tyrosinase inhibition activity of *Impatiens balsamina* flowers[7].

Conclusion

Impatiens balsemina.L seeds were collected and extracted and screneed for Phytochemical analysis. Phytochemical analysis of the extract revealed the presence of alkaloid, flavonoid, terpenoid and tannins. The extract of *Impatiens balsemina*.L was also subjected to antioxidant, antiinflammatory and andtidiabetic activity. Our *in vitro* research indicated that the extract had intensive inhibition activity for BSA, with an IC50 of cell inhibition effect of 6.08 ± 0.08 mg/L. Our work implies that the Chinese herb *Impatiens balsamina* does indeed have anti-diabetic bioactivities. This finding will greatly benefit the clinical use of *Impatiens balsamina.*

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Conflict of Interest

Authors declare no conflict of interest.

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