Phytochemical Analysis and Evaluation of Anti-inflammatory Activity of *Bignonia venusta (*Ker Gawl.) Miers Flower Extracts

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Abstract-The inflammatory response involves a complex array of enzyme activation, mediator release, fluid extra vasations, cell migration, tissue breakdown and repair which are aimed at host defence and usually activated in disease condition. Currently much interest has been shown in the searching of medicinal plants with anti-inflammatory activity which may lead to the discovery of new therapeutic agent that is not only used to suppress the inflammation but also used in diverse disease conditions where the inflammation response is amplifying in the disease process. In the present study, the selection of *Bignonia venusta* plant for evaluation was based on its traditional usages. Preparation of different extracts from non polar and polar solvent were prepared to study the phytochemical analysis in different extracts and anti inflammatory activities of different extracts. Methanol extract was found to be the richest extract for phytoconstituents and from the comparison with the standard drug aspirin, it was observed that the concentration of 2000 µg/ml of methanol extract showed maximum activity (58.0%) at 560 nm while the other extract from petroleum ether, in comparison with standard drug aspirin shows no activity.

Keywords: Phytochemical Analysis, Anti-inflammatory, *Bignonia venusta*

Introduction

Many species belonging to the Bignoniaceae family, such as *Bignonia venustata,* also known as *Pyrostegia venusta* (Ker Gawl). Miers are known to be of medicinal value[2]. In folk medicine, the aerial parts of *B. venusta* are mainly used as an infusion or decoction.

Traditionally, many diseases like dysentery, immoderate menstrual flow, common diseases of the respiratory system, and for the treatment of genital infections, *Pyrostegia venusta (*Ker Gawl.) Miers is used as a medicine. Diseases like diarrhea, vitiligo and jaundice are controlled by general tonic[3, 9]. Tonics made from the stem of *P. venusta* are useful for treating diarrhea, where as flower preparation has been showed to attenuate vomiting[13]. The decoction of aerial parts of *P. venusta* is used for the treatment of cough and flu by local Brazalians. It was shown by Immuno-Modulatory study of the methanol extract of flowers of *P. venusta* that it stimulates the immune system. It supports increase in anti inflammatory and

suppress pro- inflammatory cytokines. *P. Venusta* is a natural source of phytochemicals like terpenoids, alkaloids, tannins, steroids, and saponins which has been been correlated with potential degrees of anti-inflammatory and analgesic activity[1]. Compounds like β-sitosterol, n-hentriacontane, acacetin-7-O-β-glucopyranoside and meso-inositol having anti-inflammatory activities [8]. LPS is responsible for sickness behavior due to production of pro-inflammatory cytokines which provokes a number of neuropsychological symptoms [5]. Many studies have showed that acacetin inhibits the induction of nitric oxide synthase (NOS) and cyclooxygenase-2 (COX-2) in macrophages that are activated with LPS by inhibiting the transcriptional activation [6]. The objective of this study was to evaluate the phytochemical constituents and anti-inflammatory effects of the methanolic and petroleum ether extract of *B. venusta* flower.

Material and Methods

Experimental work was carried out under following headings on the flowers of *Bignonia venusta* for their anti-inflammatory activity.

- Collection and identification of flowers of *Bignonia venusta*
- Extraction of flowers of *Bignonia venusta* in non-polar and polar solvents.
- Phytochemical analysis and thin layer chromatography of different extracts.
- Anti-inflammatory activity of different extracts.

Qualitative Phytochemical Tests

The different extracts made from the flowers of *Bignonia venusta* were tested for the various components as follows.

Test for alkaloids

Small portion of solvent free extract was stirred with few drops of dilute HCl and filtered. The filtrate was then tested for the following color tests.

Mayer's Test: (a) 1.36 grams of mercuric chloride was dissolved in 60ml distilled water .(b) 5gm of potassium iodide was dissolved in 20ml of distilled water. (a) and (b) were mixed and the volume was adjusted to 100ml with distilled water. Appearance of cream color precipitate with Mayer's regents showed the presence of alkaloids.

Wagner's Test: 1.27 grams of iodine and 2 grams of potassium iodide was dissolved in 5ml of water and made up to the volume to 100ml with distilled water. Appearance of reddish brown precipitate with Wagner's reagent showed the presence of alkaloid.

Hager's Test: Took 20ml of saturated solution of picric acid added few drops of it to 2-3ml of extract. A yellow color was absorbed.

Detection for carbohydrates and glycosides

Molisch's Test: 10 grams of alphe naphthol was dissolved in 100ml of 95% alcohol. Extract was treated with this solution and 0.2ml of concentrated sulphuric acid was slowly added through the side of the test tube, purple or violet color appeared at the junction. This indicated the presence of carbohydrates and glycosides.

Benedicts Test: The test solution was treated with few drops of Benedict's solution (alkaline solution containing cupric citrate complex) and upon boiling on water bath, reddish brown precipitate formed, showing reducing sugars were present.

Fehling's Test: 6.932 grams of copper sulphate was dissolved in distilled water and make volume upto 100ml (solution a). 34.6 grams of potassium sodium tartarate and 10 grams of sodium hydroxide was dissolved in distilled water and make volume upto 100 ml (solution b). two solutions were mixed in equal volume prior to use and few drops of sample were added and boiled, a brick red precipitate of cuprous oxide were formed, if reducing sugar were present.

Barfoed's test: 16.5 grams of copper acetate was dissolved in 24 ml of water and 2.5 ml of glacial acetic acid was added to it. Reddish brown precipitate were formed on boiling if reducing sugar were present.

Test for Sterols and Triterpenoids

Salkowski Test: Extract was treated with few deops of concentrated suphuric acid, shake well and allow to stand for some time, red color appear at the lower layer indicated the presence of steroids and formation of yellow coloured lower layer indicated the presence of triterpenoids.

Sulphur Powder Test: Small amount of sulphur powder was added to the test solution, it sinks at the bottom, showing presence of sulphur powder.

Test for Proteins and Amino acids

Biuret Test: To 3 ml test solution 4 % w/v NaOH and few drops of 1% w/v copper sulphate solution were added. A blue color was absorbed.

Ninhydrin Test: 1 gram of ninhydrin (indane 1,2,3 trione hydrate) was dissolved in n-butanol and made the volume 200 ml. Extract treated with this solution gave violet color on boiling.

Test for saponins

Foam Test: 1ml of extract was diluted with distilled water to 20 ml and shook in a graduated cylinder for 15 minutes. A 1 cm layer of foam indicated the presence of saponins.

Test for Tannins and Phenolic compounds

Ferric Chloride test: Extract was treated with ferric chloride solution, blue color was appeared if hydrlysable tannin was present and green color was appeared if condensed tannins were present.

Vanillin Hydrochloride Test: 1 gram vanillin was dissolved in 10 ml alcohol and 10 ml concentrated hydrochloride solution. Extract was treated with this solution gave pink or red color due to the presence of tannins and phenolic compounds.

Anti-inflammatory Activity of extract

There are several methods to determine anti-inflammatory activity. In the present study, we had studied the anti-inflammatory activity by Hypotonic induced hemolysis method.

Preparation of NIH solution:

Trisodium citrate	-	5.5 gm
Citric acid	-	2.0 gm
Dextrose	-	6.125 gm
Distilled water	-	250 ml

Used 0.8 ml of NIH solution to preserve 5 ml of blood [14]

Effect of Hemolysis [11]

Erythrocyte suspension

Whole blood was collected from goat under ether anaesthesia. Heparin was used to prevent clotting. The blood was washed three times with 0.9% saline. The volume of saline was measured and reconstituted as a 40% (v/v) suspension with isotonic buffer solution (Ph 7.4).which was contained in 100ml of distilled water. The isotonic buffer solution was composed of 154mm NaCl in 10mm sodium phosphate buffer (pH 7.4).

Hypotonic solution-induced hemolysis:

Stock erythrocyte suspension was mixed with hypotonic solution containing the *bignonia venusta* flower extract at different concentrations, while the control was kept drug free. They were incubated for 10 min at room temperature and centrifuged at 3000g for 10 min. All the experiments were performed in triplicates and the absorbance (O.D) of the supernatant was measured at 560nm.

Acetyl salicylic acid (200mg/ml) was used as a reference standard.

Calculation

The percentage inhibition or acceleration of hemolysis in tests was calculated according to the equation:

% acceleration or inhibition of hemolysis = 100*(OD1-OD2/OD1)

Where, OD 1 = optical density of hypotonic saline solution + blood (control) and OD 2 = Optical density of test sample in hypotonic saline solution + blood.

Results and Discussion

Phytochemical Analysis

The extract of flowers of *Bignonia venusta* undergoes various qualitative chemical tests. They showed their presence and absence in the both solvent system which is summarized in table-1. From the table, we can find out that methanol extract was the richest extract for phytoconstituents except proteins and amino acids. It contains all tested phytoconstituents viz. Alkaloids, carbohydrates, saponins and phenolic compounds.

The studied flowers were extracted by two solvents i.e. petroleum ether and methanol by cold percolations and the yield of flower extracts in petroleum ether and methanol are 35 ml and 3.45 gm respectively.

In a study on the extracts of leaves of *Bauhinia variegata*, it was reported that the methanol extract was richest extract for phytoconstituents[4]. Except tannins of phenolic compounds, carbohydrates and flavonoids, it

contains all tested phytoconstituents viz. Alkaloids, glycosides, proteins and amino acid, triterpenoids of sterols, phenolic compounds and saponins and fats and fixed oil. The methanol extract was the richest extract for phytoconstituents while petroleum ether extract contains least phytoconstituents was reported on the study on the extracts of seeds of *Gmelina arborea*[10]. It was also reported that the methanol extracts of the leaves and flowers of *Cassia glauca* was the richest source of phytochemicals [12]

The anti-inflammatory activity of the different extracts of *Bauhinia variegata* flowers was compared with activity of standard drug Aspirin at 560 nm. From the comparison with the standard drug, it was observed that the concentration of 2000 µg/ml of methanol extract showed maximum activity (58.0%) at 560 nm while the other extract from Petroleum ether, in comparison with standard drug aspirin shows no activity. Table(2,3 and 4).

In an author work the anti-inflammatory activity of the different sample extracts of leaves of *Bauhinia variegata* was also compared with activity of standard drug Aspirin at 560 nm. From the comparisonWith standard drug it was observed that the n-Butanol extract shows maximum activity at 560 nm while other extract such as methanol, chloroform and petroleum ether show less activity[4]. In his study, he reported that petroleum ether extract showed more anti-inflammatory activity in comparison to that of methanol extracts of leaves of *Bauhinia variegata.*

It was also reported in a study on the anti-inflammatory activity of the different sample extracts of seeds of *Gmelina arborea* that methanol extract showed more anti-inflammatory activity at 560 nm in comparison to standard drug i.e. Aspirin while other extract of petroleum ether show less activity [10].

Singh[12] worked on the anti-inflammatory activity of the leaves and flower extracts of *Cassia glauca*. He compared the anti-inflammatory activity of the different sample extracts with the activity of standard drugAspirin at 560nm. From the study, he reported that the concentration of 3000 µg/ml of methanol extract showed the maximum activity at 560nm, as compared to acetone, chloroform and hexane[12].

Table-1 Qualitative Phytochemical analysis of extract of *Bignonia venusta* flower.

Test performed	Pet. Ether Extract	Methanol Extract
Test for Alkaloids		
Mayer's test	-	-
Hager's test	-	+
Wagner's test	-	+
Test for carbohydrates		
Fehling's test	-	-
Molish's test	-	+
Benedict test	-	+
Barfoed's test	-	+
Test for Steroids		
Salkowaski test	+	+
Test for Saponins		
Foam test	-	+
Test for phenolic compounds		
$Fecl_3$ - test	-	+
Vanillin HCl test	+	+
Test for proteins and Amino acids		
Biuret's test	-	-
Ninhydrin test	-	-

(-) Absence, (+) Presence

Table-2 Standard drug used for Anti-inflammatory action and results

Sample Aspirin	concentration	Absorbance (at 560nm)	% Inhibition of hemolysis
1	Control	0.711	-
2	1000	0.359	49
3	1500	0.323	54
4	2000	0.296	58

Table-3 Effect of different extracts of bignonia on stability of erythrocyte membrane: (petroleum ether extract).

Sample	Concentration (µg/ml)	Absorbance (at 560nm)	% Inhibition of hemolysis
1	Control	1.72	-
2	1000	1.92	0
3	1500	2.69	0
4	2000	2.08	0

Table-4 Effect of different extracts of *Bignonia venusta* on stability of erythrocyte membrane: (Methanol extract).

Sample	Concentration (µg/ml)	Absorbance (at 560nm)	% Inhibition of hemolysis
1	Control	1.47	-
2	1000	1.28	12
3	1500	1.17	20.4
4	2000	1.09	25.85

Conclusion

The anti-inflammatory activity of the different extracts *Bignonia venusta* was compared with the activity of standard drug Aspirin at 560nm. From the comparison with the standard drug, it was observed that concentration of 2000µg/ml of methanol extract showed maximum activity (58.0%) at 560 nm while the other extract from petroleum ether, in comparison with standard drug aspirin shows no activity. The methanol extract of *Bignonia venusta* showed increase in the protection of the erythrocyte membrane against hypotonic haemolysis and less protection shown by petroleum ether.

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