ISSN 2278- 4136
ZDB-Number: 2668735-5
IC Journal No: 8192
Volume 1 Issue 4
Online Available at www.phytojournal.com
Journal of Pharmacognosy and Phytochemistry

In-Vitro Thrombolytic and Anti-inflammatory Activity of *Swertia chirata* Ethanolic Extract.

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Ethanol extract of *Swertia chirata* was assessed for its thrombolytic, anti-inflammatory activity and phytochemical screening. *In vitro* anti-inflammatory activity was evaluated using albumin denaturation. Aspirin was used as a standard drug for the study of anti-inflammatory activity. The ethanol extract of *Swertia chirata* showed mean inhibition of protein denaturation 45.31±0.000576 whereas, for control group it was found to be 50.00±0.00177. In thrombolytic activity using *in vitro* clot lysis assay method, the crude ethanol extract was found to have significant, thrombolytic test showed a maximum effect of 40.38% while the standard streptokinase showed 69.35.

Keyword: Swertia chirata, Thrombolytic, Anti-inflammatory.

1. Introduction

Medicinal plants have always been considered a healthy source of life for all people. Therapeutically properties of medical plants are very useful in healing various diseases and the advantage of these medicinal plants is being 100% natural.

Nowadays people are being bombarded with thousands of unhealthy products, the level of sensibility in front of diseases is very high and that's why the use of medicinal plants can represent the best solution. Since antiquity, man has used plants to treat common infectious diseases and even long before mankind discovered the existence of microbes; the idea that certain plants had healing potential was well accepted[1]. A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs. A number of plants have been used in traditional medicine for many years due to their antimicrobial properties[2]. Specifically, the medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human or animal body[3]. The most important of these bioactive constituents which are mainly secondary metabolites are alkaloids, flavonoids, tannins and phenolic compounds. These phytochemicals are toxic to microbial cells.

Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act

as an etiologic factor for many of these chronic illnesses. Although it is a defense mechanism, the complex events and mediators involved the inflammatory reaction can induce, maintain or aggravate many diseases. Currently used synthetic anti-inflammatory drugs are associated with some severe side effects. Therefore, the development of potent anti-inflammatory drugs with fewer side effects is necessary from medicinal plants origin.

A blood clot (thrombus) developed in the circulatory system due to failure of hemostasis causes vascular blockage and while recovering leads to serious consequences in atherothrombotic diseases such as myocardial or cerebral infarction, at times leading to death.[5] Thrombolytic agents that include tissue plasminogen activator (t-PA), Urokinase (UK), streptokinase (SK) etc. are used all over the world for the treatment of these diseases. In India, though SK and UK are widely used due to lower cost,[6,7] as compared to other thrombolytic drugs, their use is associated with hyper risk of hemorrhage[8] severe anaphylactic reaction and lacks specificity. Moreover, as a result of immunogenicity multiple treatments with SK in a given patient are restricted[9]. Because of the shortcomings of the available thrombolytic drugs, attempts are underway to develop improved recombinant variants of these drugs[10 – 14].

Herbal products are often perceived as safe because they are "natural".[15] In India, in recent years, there is increased research on traditional ayurvedic herbal medicines on the basis of their known effectiveness in the treatment of ailments for which they have been traditionally applied.
Considerable efforts have been directed towards the discovery and development of natural products from various plant and animal sources which have antiplatelet[16, 17], anticoagulant,[18, 19] antithrombotic [20], and thrombolytic activity. Epidemiologic studies have provided evidence that foods with experimentally proved antithrombotic effect could reduce risk of thrombosis. Herbs showing thrombolytic activity have been studied and some significant observations have been reported[21].

The aim of present study was to screen extracts of *Swertia chirata* for its clot lysis property (thrombolytic activity) and anti-inflammatory activity by using an *in-vitro* procedure. Plant-derived drugs remain an important resource, especially in developing countries, to combat serious diseases. Approximately 60–80% of the world's population still relies on traditional medicines for the treatment of common illnesses. Medicinal plants have a long-standing history in many locations in Bangladesh and continue to provide useful and applicable tools for treating ailments. Nevertheless, little scientific research was done to investigate the plants *Swertia chirata.*

The activities have been selected because of their great medicinal relevance. Within the recent years, Heart diseases have increased to a great extent and side effects of synthetic drug becomes an ever-increasing therapeutic problem [4] Because natural products of higher plants may give a new source of thrombolytic agents, as well as anti-inflammatory agents, many research groups are now engaged in medicinal plants research.

2. Materials and methods

2.1 Plant Material

The plant *Swertia chirata* was selected based on variety of its medicinal value. The *Swertia chirata* was collected at their fully mature form, from local market (Khatunghang, Chittagong). It was then separated, cleaned from impurities.

2.2 Extraction & Preparation of the plant sample

The plant *Swertia chirata* was then subjected for shade dry at temperature not exceeding 50 ºC. Then they were ground into coarse powder with help of a grinder. The dry powder was then subjected to cold extraction with ethanol and then fractionation by Chloroform and Petroleum ether.

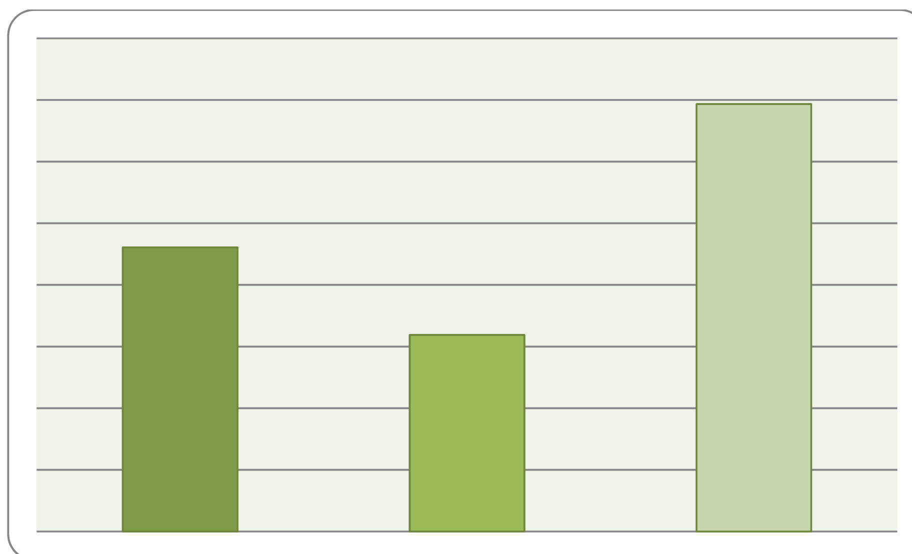

Fig 1: Percent clot lysis of Ethanol extract, Chloroform fraction of *Swertia chirata* and Standard Streptokinase.

2.3 *In vitro* Thrombolysis Activity

Phosphate buffered saline (PBS) (5 ml) was added to the commercially available lyophilized streptokinase vial (15, 00,000 I.U.) and mixed properly. This suspension was used as a stock from which appropriate dilutions were made to observe the thrombolytic activity. Experiments for clot lysis were carried as reported.[22] In brief; 2 ml venous blood drawn from healthy volunteers was distributed in three different pre weighed sterile micro centrifuge tube (0.5 ml/tube) and incubated at 37 °C for 45 minutes. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed) and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone). To each micro centrifuge tube containing pre-weighed clot, 100 µl of ethanol extract (10 mg/ml) of was added. As a positive control, 100 µl of streptokinase and as a negative non-thrombolytic control, 100 µl of distilled water were separately added to the control tubes numbered. All the tubes were then incubated at 37 °C for 90 minutes and observed for clot lysis. After incubation, fluid released was removed and tubes were again weighed to observe the difference weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as constant oxygen supply for 48 hours.

2.4 *In-vitro* Anti-inflammatory activity

The ethanol extract of *Swertia chirata* was screened for anti-inflammatory activity using inhibition of albumin denaturation technique which was studied according to Mizushima and Kobayashi with slight modification.[23, 24, 25] The standard drug and extract were dissolved in minimum quantity of dimethylformamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solution was less than 2.5%. Test solution (1ml) containing different concentrations of drug was mixed with 1 ml of 1mM albumin solution in phosphate buffer and incubated at 27±1 °C in BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60±1 °C in water bath for 10 min. After cooling, the turbidity was measured at 660 nm. Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average is taken. The Acetyl Salicylic Acid was used as standard drug. The percentage inhibition of denaturation was calculated by using following formula.

% of Inhibition = 100 X { Vt / Vc – 1 }

Where,
Vt = Mean absorbance of test sample.
Vc = Mean absorbance of control

3. Results

3.1 Result of Thrombolytic Activity

In thrombolytic activity using *in vitro* clot lysis assay method, the crude ethanol extract and chloroform fraction showed average clot lysis of 46.096% and 31.87% while the standard streptokinase showed 69.35% which is shown in Figure-1. The percentage of clot lysis was found to be significant when compared with the vehicle control.

3.2 Result of Anti-inflammatory activity

Anti-inflammatory activity was measured by measuring the absorbance of treatment groups and converting it into total inhibition of protein denaturation. The statistical data were obtained significant below the P-value<0.5 ($p<0.001$). Percent inhibition of protein denaturation was calculated as follows:

$$\%Inhibition = (Abs_{control} - Abs_{sample})/Abs_{control} \times 100.$$

In the present study for *in-vitro* anti-inflammatory test, the 1000 mg/kg of crude ethanol extract of *Swertia chirata* showed mean inhibition of protein denaturation 45.31±0.000576 and whereas for Acetyl Salicylic Acid, it was found to be 50.00±0.00177.

Table 1: *In- vitro* of anti-inflammatory activity of *Swertia chirata*

Observation	Mean Absorbance ±SD	%MIPD	Total Inhibition of Protein Denaturation (%MIPD±SEM)
ETHANOL (Vehicle Control)	0.064±0.00416	0	0.00±0.00294
ASA (Positive control)	0.032±0.00416	50	50.00±0.00177
EESC 1000mg/kg	0.035±0.00416	45.31	45.31±0.000576

ASA= Acetyl Salicylic acid,
MIPD= Mean inhibition of Protein denaturation,
EESC= Ethanol Extract of *Swertia chirata.*
SEM= Standard error of mean,
Total inhibition of protein denaturation= %MIPD±SEM,[a]$p<0.001$

Fig 2: % MIPD of Ethanol, ASA and EESC.

4. Discussion and conclusion

The result of this work showed that the extract of *Swertia chirata* had mild to moderate ani-inflammatory activity (Table 1). The results of clot lysis was indicated that test samples showed different thrombolytic activity e at different concentration. The clot lysis of *Swertia chirata* was found to be increased with the increase with the concentration of the sample. The significant average percent of clot lysis (46.096%) of ethanol extract of *Swertia chirata* was found. Therefore, it is evident that the test sample and ethanol extract were thrombolytic and possess anti-inflammatory activity as well as biologically active.

In conclusion, it can be claimed that *Swertia chirata* possesses significant anti-inflammatory activity as well as thrombolytic activity. In addition, positive result in thrombolytic activity test led us to the interference that the plant extract may contain bioactive compounds, which may aid ongoing cardiovascular drug discovery from the floristic resources. Hence, further studies are suggested to be undertaken to pin point the exact compounds and to better, understand its actions scientifically.

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