"In-vitro examination of extracts obtained from leaves of *Spathodea companulata*"

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Abstract

Medicinal plants are gift of God having answers for almost all type of diseases. Since ancient time in India many healers used these medicinal plants in treatment of various pathogenesis. Now a days this knowledge is accepted globally. Hurdle in establishing the concept, is proper characterization of these medicinal plants on the basis of physical, chemical and biological properties. Among these medicinal plants, many are reported to have significant effect on various systems and disease. These plants possess great therapeutic effects on human body and can be good cure to many deadly diseases. The plant *spathodea campanulata* falls under such category. In the present research work the leaves have been studied. These are first identified and the leaf extracts obtained from ethyl acetate, chloroform, petroleum ether and ethanol. The extracts were obtained and were later tested for in-vitro antiinflammatory studies in the animals.

Keywords- spathodea campanulata, anti-inflammatory activity,

Introduction

Spathodea campanulata L. is native of tropical Africa, with orange scarlet bell shaped flowers, three by two and half inch large, that appear in November, the climate of Mumbai seems to suit it and may be seen in full flowering in the month of November. It is generally planted as an ornamental plant along the roadside. Hence, the flowers are easily available and abundant. The flower part is most colourful and consists of maximum amount of chromophores responsible for the activity. Genus name comes from the Greek words *spathe* meaning sheath and *oides* meaning resembling in reference to calyx shape.

Phytochemical studies yield alkaloids, tannin, saponin, steroids, terpenonids, flavonoids. Study to analyze the constituents of the flower yielded four compounds. Butane, 1, 1-diethoxy-3-methyl- (35.11%) and n-Hexadecanoic acid (30.22%) were the major constituents of the ethanolic extract. Stem bark has yielded spathodic acid, steroids, saponins, ursolic acid, tomentosolic acid and pectic substances. Phytochemical screening phytochemical screening yielded carbohydrates, alkaloids, tannins, glycosides in the extracts of flowers and steroids, carbohydrates, alkaloids, tannins and glycosides in the bark.

Traditional uses

The bark and leaves are widely used in traditional medicine in Ghana. The bark is used in wound healing and especially burn healing. The bark and leaves shows a wide spectrum of antibacterial activity including antimalarial activity. Aqueous alcoholic decoctions of the leaves shows promise to be used for the treatment of

malaria. The stem bark decoction has shown hypoglycemic activity in mice. Ethanol leaf extracts show anticonvulsant activity. African tulip tree is planted as an ornamental, a wayside tree and shade tree. It is used for soil improvement, reforestation, erosion control and land rehabilitation, and as a live fence. The seeds are edible. The soft white timber used in making paper and a wood is used to make drums. The bark, flowers and leaves are used in traditional medicines in Western Africa. The seeds are eaten in many parts of Africa. The flower buds contain a reddish sap and are used as water pistols by children. In Ethiopia, it is used as firewood and to produce charcoal.

Medicinal uses (huo Yan Shu, 2012)

Spathodea campanulata has many medicinal uses both where it is native and introduced. Extracts of bark, leaves and flowers are used to treat malaria, HIV, diabetes, meliitus, Oedema, dysentery, constipation, gastrointestinal disorders, ulcers, skin diseases, wounds fever, urethral inflammation, liver complaints and as a poison antidote. It may be effective as malaria prophylactic and in the control of *Aedes* Mosquitoes. The bark has laxative and antiseptic properties and the seeds, flowers and roots are used as medicine. The bark is chewed and sprayed over swollen cheeks. The bark also is boiled in water used for bathing newly born babies to heal body rashes. Bark is commonly used as a dressing for ulcers and skin diseases, applied dried, pulverized, or as fresh inner bark. A leaf decoction is used as lotion. In Africa, the stem bark is used as a paste for wound healing. In Senegal, bruised leaves and flowers are applied to wounds. In Gabon, flowers are applied to ulcers. In Southern Nigeria, leaves used for convulsions. In Gold Coast, bark decoction taken for constipation and gastrointestinal problems and dysentery. Cold infusion of leaves used for urethral inflammation. In Ghana, the stem bark and leaf used for treatment of dyspepsia and peptic ulcer; leaf, root bark and fruit used for arthritis and fractures; the stem bark used for toothaches and stomachaches; root bark seed used for stomach ulcers. In Rwanda decoction of stem bark used for diabetes. In Ayurveda it is used for kidney diseases.

MATERIAL AND METHODS

Spathodea companulata was collected and authenticated by Dr. Zia-Ul-Hasan, HOD, Department of Botany. The parts were then washed thoroughly and then they were extracted in different solvents and further specific phytochemical constituent isolation was done.

In-vitro anti-inflammatory activity

A) Hypotonic solution –induced haemolysis or membrane stabilizing activity:

This test was done according to the method described by Shinde et al., 1999 with slight modifications. The test sample consisted of stock erythrocyte (RBC) suspension 0.03 ml mixed with 5 ml of hypotonic solution (154 mM NaCl in 10 mM Sodium Phosphate Buffer at pH 7.4) containing Herbal Preparation ranging from concentration 100-500 µg/ml. The control sample consisted of 0.03 ml RBC suspension mixed with hypotonic buffered solution alone. The standard drug acetylsalicylic acid was treated similar to test at 100 and 200 µg/ml

concentrations. The experiment was carried out in triplicate. The mixtures were incubated at 10 minutes at room temperature, centrifuged for 10 minutes at 3000rpm and absorbance of the supernatant was measured spectrophotometrically at 540 nm. The percentage inhibition of haemolysis or membrane stabilization was calculated by following equation.

% Inhibition of haemolysis = 100 x [A 1 –A 2 /A 1]

Where:

A 1 = Absorbance of hypotonic buffered solution alone

A 2 = Absorbance of test /standard sample in hypotonic solution.

B) Effect on Protein Denaturation:

Protein denaturation was performed as described by Elias et al., **1988** with slight modifications. Test solution consisting of 1ml of different concentrations of Herbal Preparation ranging from 100-500 µg/ml or standard acetylsalicylic acid 100 and 200 µg/ml was mixed with 1ml of egg albumin solution (1 mM) and incubated at 27 ±1˚C for 15 minutes. Denaturation was induced by keeping the reaction mixture at 70˚C in a water bath for 10 minutes .After cooling the turbidity was measured spectrophotometrically at 660 nm. Percentage inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and the average was taken.

The percentage inhibition of haemolysis or membrane stabilization was calculated by following equation.

% Inhibition of haemolysis = 100 x [A 1 –A 2 /A 1]

RESULTS AND DISCUSSION

In-vitro Anti-inflammation Hypotonic:

The membrane stabilization by hypotonic solution-induced haemolysis method was used to assess antiinflammatory activity of the plant extracts by following standard protocol. Since the erythrocyte membrane resembles to lysosomal membrane and as such, the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane. The membrane stabilizing activity of the extractives was assessed by using hypotonic solution-induced human erythrocyte haemolysis.

SC Leaves

Conc.	**STD**	**CH**	**EA**	**ALC**
100 µg/ml	38.420	1.226	5.751	8.435
200 µg/ml	43.404	2.453	7.515	12.116
300 µg/ml	47.776	3.450	10.199	16.717
400 µg/ml	52.990	4.601	12.269	20.552
500 µg/ml	59.585	6.671	13.650	22.699

The percentage inhibition of haemolysis was studied in order to study the anti-inflammatory activity of the extracts. Haemolysis was induced through hypotonic solution. The anti-inflammatory activity of extraction of alcohol, chloroform and ethylacetate was compared with the values of standard. The extracts were given in increasing concentration of dose of the drug in order to study anti-inflammatory action. The number of erythrocytes surviving after each administration was calculated through percentage inhibition of haemolysis. Upon comparison, the maximum number of erythrocytes surviving was found in the extract of alcohol. The anti-inflammatory activity increased further upon increasing the dose of the drug in the subjects. Maximum activity was shown by extracts of alcohol followed by ethylacetate and chloroform. Alcoholic extracts showed maximum activity.

In-vitro Inflammation Denaturation:

Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well-documented cause of inflammation.

SC Leaves

Conc.	**STD**	**CH**	**EA**	**ALC**
100 µg/ml	68.149	27.046	30.782	35.053
200 µg/ml	73.843	30.427	35.587	37.900
300 µg/ml	80.071	35.587	39.679	43.594
400 µg/ml	82.562	43.060	46.085	46.975
500 µg/ml	85.231	48.220	48.754	52.491

In-vitro inflammation denaturation was studied in order to get a view of anti-inflammatory activity of the extracts. The higher the denaturation more is the inflammation and vice-versa. In this in-vitro test, percentage inhibition was studied. The extracts in chloroform, ethyl acetate and alcohol were studied for the study and obtained results were compared to the standard. Amongst the values obtained the alcoholic extract shows maximum activity when compared with the standard followed by chloroform and ethyl acetate.

IAEC Approval

All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal (Reg No. 1824/PO/ERe/S/15/CPCSEA). Protocol Approval Reference No. PBRI/IAEC/PN-17033.

Animal used *Albino wistar rats*

Weight 200±25 gm

Sex	Either
Route of administration	P.O.
Housing Condition	Animals were housed in separate cages under controlled conditions of temperature (22 ± 2°C). All animals were given standard diet (Golden feed, New Delhi) and water regularly.

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