

# In-vitro anti inflammatory examination of extracts obtained from bark of *Spathodeacompanulata*

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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 *spathodeacompanulata* falls under such category. In the present research extracts of bark have been studied. These are first identified and the bark extracts have been tested for several therapeutic effects. The bark was put through extraction from petroleum ether, methanol, chloroform, ethyl acetate. The extracts were later tested for in-vitro anti-inflammatory activities in the animals.

## 1. Introduction

*Spathodeacompanulata* 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

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

*Spathodeacompanulata* 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. 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 Gold Coast, bark decoction taken for constipation and gastrointestinal problems and dysentery. 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 (huo Yan Shu, 2012).

## 2. Material and methods

*Spathodeacompanulata* was collected and authenticated by Dr. Zia-UI-Hasan, HOD, Department of Botany. The parts where extraction was to be performed were washed under running tap water and kept in shade for drying. They were later pulverized using mechanical grinder. They were then packed in labelled containers. Then they were subjected to extraction using hot percolation 'Soxhlation'. Pulverised dried material of *Spathodeacompanulata* were placed in thimble of Soxhlet apparatus. Extraction of *Spathodeacompanulata* was done with percolation method. Extraction was performed at 60°C using

petroleum ether as non-polar solvent at first. Exhausted plant material (mark) was dried and afterward extracted with ethyl acetate, chloroform and methanol. Obtained extracts were evaporated and using rotary vacuum evaporator (Buchi type) at 40°C. These extracts were then tested for in-vitro anti-inflammatory activity through hypotonic solution-induced haemolysis or membrane stabilising activity and effect on denaturation.

### 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 mMNaCl 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.

$$\% \text{ Inhibition of haemolysis} = 100 \times [A_1 - A_2 / A_1]$$

Where:

A<sub>1</sub> = Absorbance of hypotonic buffered solution alone

A<sub>2</sub> = 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.

$$\% \text{ Inhibition of haemolysis} = 100 \times [A_1 - A_2 / A_1]$$

### 3. 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 Bark				
Conc.	STD	CH	EA	ALC
100 µg/ml	38.420	6.901	9.432	13.420
200 µg/ml	43.404	8.665	11.503	16.871
300 µg/ml	47.776	10.812	13.190	20.935
400 µg/ml	52.990	12.269	15.107	23.926
500 µg/ml	59.585	13.420	16.564	27.337

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 were 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.

#### 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 BARK				
Conc.	STD	CH	EA	ALC
100 µg/ml	68.149	36.654	50.533	52.846
200 µg/ml	73.843	41.814	56.761	60.676
300 µg/ml	80.071	48.932	60.320	64.234
400 µg/ml	82.562	56.583	64.056	65.124
500 µg/ml	85.231	58.540	65.836	66.725

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 table the percentage inhibition of protein denaturation was studied in order to study the anti-inflammatory activity of the extracts. 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. Upon comparison, the maximum percentage of inhibition of denaturation 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.

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