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RESEARCH ARTICLE

Studies on Phytochemical Screening and *in- vitro* anti-inflammatory activity of *Delonix elata* L. leaves

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Abstract

Inflammatory diseases including arthritis and rheumatism are major group of prevalent diseases. Inflammation is the complex biological response of vascular tissues to harmful stimuli including pathogens, irritants or damaged cells. Many substances, interfering with the inflammatory response have been isolated from medicinal plants. Natural products with anti inflammatory activity mainly contain active principles viz, flavonoids, terpenoids, steroids, Phenolic compounds, saponins and alkaloids. The aim of this study is to investigate the Phytochemical constituents and evaluated for possible anti-inflammatory activity of leaves of *Delonix elata* by human red blood cell (HRBC) membrane stabilization method. The extract of leaves of *D. elata* contains terpenoids, flavanoids, steroids, phenols, cardioglycosides, quinines, coumarins and tannins. The ethanolic extract of leaves of *D.elata* is evaluated for anti-inflammatory activity by human red blood cell (HRBC) membrane stabilization method and their activities are comparable to that of the standard drug Aspirin.

INTRODUCTION

Inflammation is protective and defense mechanism of the body. It is the reaction of vascularized living tissue to local injury. Inflammation is the complex biological response of vascular tissues to harmful stimuli including pathogens, irritants or damaged cells. (1) During inflammatory conditions various pathological changes are take place. The production of active inflammatory mediators is triggered by microbial products or by host proteins, such as proteins of the complement, kinins and coagulation systems that are themselves are activated by microbes and damaged tissues. (2, 3)

Inflammation is the normal physiological and immune response to tissue injury. Increased blood supply, enhanced vascular permeability and migration of immune cells occur at damaged sites. The inflammatory process is a protective response that occurs in response to trauma, infection, tissue injury or noxious stimuli. It can be identified by tumar (swelling); Robor (redness), Calor (heat) and Dolar (pain) (4, 5, 6).

Delonix elata is a small sized tree found in Gujarat, western peninsular and southern India belonging to the family Fabaceae – Caesalpinioideae. Under *Delonix* genera three species of tropical origin are present. Two variants are present in eastern part of Africa. *Delonix* is derived from Greek word Delos, which means a claw that looks like petals and *elata* means tall. It is commonly called creamy peacock flower, flamboyant tree, tiger bean, white gul mohur in English, sandesaro in Gujarati, flamboyant in French, mseele in Swahili, padenarayan or pandenarayan in Tamil. It is not classically used as Ayurvedic drug, but is included in Shodhala Nighantu with Sanskrit name "Siddeshwara" (7-9). In India and china, the bark extract of this plant is considered as febrifuge and anti-periodic. The leaf and bark in the form of paste is used by local traditional medicinal practitioners to reduce inflammation and pain (10).

The medical usefulness of the tree is acknowledged by people living in the villages who take a decoction of the leaves and barks to get relief from rheumatic problems like pain and stiffness of the joints, especially affecting the knees (11, 12). It was observed that local people and Siddha practitioners in Tamil Nadu, India use the *Delonix elata* bark and leaves for treating inflammation and arthritic conditions. The benefits may be attributed to the chemical constituents like ß-sitosterol, quercetin, lupelol, lysine, alanine, valine, tyrosine and rhamnose are which reported from *Delonix regia.* Quercetin 3-O-rhamnoglucoside and Quercetin-3O-galactoside are also reported (13). Extensive pharmacological studies on *Delonix elata* exhibited anti-inflammatory (12, 14, 15, 16,) and anti- arthritic activity.

MATERIALS AND METHODS

Collection of sample

Fresh plant leaves of *D.elata* were collected from different places of Chennai. The leaves were washed thoroughly with normal tap water followed by sterile distilled water. Then the leaves were shade dried at room temperature. Leaves were crushed to powder using grinding machine. The powdered sample was analysed for qualitative inorganic compounds.

Preparation of extracts

Preparation of the extracts was following the standard methods (17, 13). About 15g of fine dried powdered leaves of *D.elata* plant materials was extracted with 150mL of ethanol (75%), acetone, Chloroform, petroleum ether and aqueous extract for 1 min using an Ultra Turax mixer (13,000rpm) and soaked overnight at room temperature. The samples was then filtered through Whatman No.1 paper in Buchner funnel. The filtered solution was evaporated under vaccum in a rota-evator at 40°C to a constant weight and then dissolved in respective solvents. The concentrated extract were stored in airtight container in refrigerator below 10°C.

PRELIMINARY PHYTOCHEMICAL ANALYSIS:

Test for Tannin

1 mL of leaf extract was taken in a test tube. To that 1mL of 5% ferric chloride was added. Formation of greenish black colour indicates the presence of tannin.

Test for Saponin

To 1 mL of leaf extract was added to 2mL of distilled water in a test tube. The solution was shaken for 15minutes observed for stable persistent foam of about 0.5 to 1 cm layer indicates the presence of saponin.

Test for Flavonoid

To 1mL of 2N NaoH was added to 1mL of leaf extract. Appearance of yellow colour indicates the presence of flavonoid.

Test for Quinone

To 1mL of leaf extract 1.5mL of conc. sulphuric acid was added. The solution was observed for the formation of red colour indicates the presence of quinone.

Test for Cardioglycoside (kellerkillani test)

To 1mL of leaf extract, 2mL of glacial acetic acid and 0.5mL of 5% ferric chloride was added.To that 1.5mL of conc.sulphuric acid is added and observed for the formation of brown colour.

Test for Terpenoid (Salkowski Test)

1mL of chloroform was added to 1mL of leaf extract and 1.5mL of conc.sulphuric acid is added to it. Formation of reddish brown colour indicates the presence of Terpenoids

Test for Phenol

To 1mL of leaf extract, 1mL of sodium carbonate was added. To that 1mL of folin was added. Formation of blue or green colour indicates the presence of Phenols.

Test for Coumarin

Add 1mL of 10% Sodium hydroxide to 1mL of leaf extract.The solution was observed for the appearance of yellow colour.

Test for Steroids
To 1mL of leaf extract was added to 1mL of chloroform and 1.5mL of conc.sulphuric acid. The appearance, at the interphase, a reddish brown colour showed a positive reaction.

Test for Alkaloid
To 1mL of leaf extract, 1mL of conc. Sulphuric acid was added. To that 1mL of mayer's reagent is added. The formation of green or white precipitate was regarded as positive for the presence of alkaloids.

ANTI-INFLAMMATORY ASSAY

HRBC membrane stabilization test
HRBC membrane stabilization test was performed by the following described method (18). Fresh whole human blood (10mL) was collected and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10min and were washed three times with equal volume of normal saline. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline. The reaction mixture 2mL consists of 1mL of test sample solution and 1mL of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuged tubes containing reaction mixture were incubated in water bath 56ºC for 30min. At the end of the incubation the tubes were cooled under running tab water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. Percentage of membrane stabilization activity was calculated by the formula

Percentage inhibition = (A of Control-A of Sample)/A of Control ×100

RESULTS:

Table 1: Phytochemical screening of Leaves of *Delonix elata*

Phytochemicals	Aqueous Extract	Ethanolic Extract	Chloroform Extract	Acetone Extract	Pet-ether Extract
Tannin	-	++	-	+	-
Saponin	+	++	-	-	-
Flavanoid	+	+	+	+	-
Quinone	+	++	-	++	-
Cardioglycoside	+	++	+	++	+
Terpenoid	+	++	+	++	-
Phenol	+	++	+	++	+
Coumarin	+	+	+	+	+
Steroid	+	++	+	++	+
Alkaloid	+	++	+	+	-

(+) ***Presence of phytochemicals*** **(++) Strongly** ***Presence of phytochemicals***
(-) ***Absence of phytochemicals***

Table: 2 Effect of standard drug Aspirin on Human red blood cell membrane stabilization

Concentration (µg/ml)	Optical density value	% of inhibition
Control	1.535	-
100	0.294	81.2
200	0.281	82
300	0.226	85.6
400	0.187	88
500	0.117	92

Table 3: Effecct of *Delonix elata* leaves extract on Human red blood cell membrane stabilization

Concentration (µg/ml)	Optical density value	% of inhibition
100	1.065	32
200	0.882	32.8
300	0.543	64.6
400	0.405	73.6
500	0.300	80.4

RESULTS AND DISCUSSION

Anti-inflammatory activity of this plant is reported using human red blood cell (HRBC) membrane stabilization method. The leaf extract showed significant anti-inflammatory action but it was lower than compared to standard. Compounds like bioflavonoid are reported to produce anti-inflammatory action by decreasing capillary permeability (19). Steroids are known to produce anti-inflammatory activity. The extracts tested might contains flavonoids, steriods and terpenoids which resulted in producing anti-inflammatory activity (20).

The results showed a predominant presence of tannins, flavonoids, cardioglycoside, terpenoid, phenols, steroids, alkaloids, quionones and coumarins and the results obtained from *invitro* studies clearly indicate that the ethanolic extracts of leaves of *D.elata* exhibited excellent membrane activity. Hence the plant has undoubtedly contributed to its traditional medicinal value. The anti-inflammatory activity of this exracts may be attributed to presence of flavonoids , steroids, terpenoids entities as shown in the preliminary phytochemical screening.

Flavonoids are a group of compounds with a wide range of biological effects, including anti-inflammatory activity. Various mechanisms have been proposed to explain the anti-inflammatory effect of flavonoids, these include inhibition of inflammatory mediators, inhibition of hydroxyl and lipid peroxide free radicals also, flavonoids have been found to be free radical scavengers. Free radicals play an important role in inhibition of synthesis of prostaglandin by inhibiting the enzyme prostaglandin synthetase. Moreover, several extracts containing flavonoids have been found to exert anti-inflammatory and antioxidant activity. (21).

Steroids are widely distributed in plants and are in a particular form of glycosides. The oleoresins fraction of *Curcuma amada linn.* (Zingeberceae) possesses significant anti-inflammatory and anti-arthritic activities. (22) Several plants containing high amounts of steroids have been shown to possess anti-inflammatory activity in several experimental anti-inflammatory activity.

The terpenoids form a group of compounds, the majority of which occur in the plant kingdom; a few terpenoids have been obtained from other sources. More terpenes have been discovered as an efficacious compound in human disease therapy and prevention. Terpenoid compound have been used to treat cancer, malaria, inflammation and a variety of infectious disease (viral and bacterial). Free radicals (superoxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hypochloric acid and peroxynitrite) produced during aerobic metabolism in the body can cause oxidative damage of amino acids, lipids, proteins and DNA (23, 24).

The *in vitro* anti-inflammatory studies demonstrated that ethanolic extract of *D.elata* had appreciable anti-inflammatory activity. The ethanolic extract at 500µl showed maximum inhibition 80.4%. The extracts was rich

many phytochemical compounds and predominant presence of flavonoids, steroids, terpenoids confirms the anti-inflammatory activity of *D.elata* leaves.

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