

Research Article

In-Vitro Antioxidant Potential of *Delonix regia* Leaves

J. Mariajancyrani^{1*}, G. Chandramohan¹, P. Saravanan², S. Saivaraj³¹Department of chemistry, A.V.V.M. Sri Pushpam College, Poondi, Thanjavur-613 503, Tamil Nadu, India.²Department of chemistry, Kings College of Engineering, Thanjavur.³Department of chemistry, Thirumalai Engineering College, Kancheepuram.

*Corresponding author

J. Mariajancyrani

Email: mariajancyrani@yahoo.in

Abstract: Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. The present study was carried out to identify the phytochemicals and evaluate antioxidant activity of chloroform extract of *Delonix regia* leaves. The antioxidant activity was determined by different methods via DPPH radical scavenging assay, Hydrogen peroxide scavenging assay and reducing power assay. The leaves of chloroform extract contain terpenoids, steroids, flavonoids and Tannins. The antioxidant activity found in DPPH method is more effective than other two methods. Thus the in-vitro studies clearly indicate that the chloroform extract of *Delonix regia* leaves shows significant antioxidant activity and also a better source of natural antioxidant, which might be helpful in preventing the process of various oxidative stresses.

Keywords: Antioxidant activity, flavonoids, terpenoids, Hydrogen peroxide, Tannins

INTRODUCTION

Plants have always been a part of medicinal science from the beginning of human civilization to the present modern world of synthetic medicines. Even in the presence of variety of effective synthetic drugs, use of medicinal plants for maintaining human health has acquired a lot of importance in the present era [1]. There is a global interest in non-synthetic, natural drugs derived from plant sources, because of low cost, non-toxic nature and availability. Many plants with antioxidant potential possess flavonoids and phenolic compounds [2]. Free radical reactions have been implicated in the pathology of many human disease including atherosclerosis, ischemic heart disease, the aging process, inflammation diabetes and other conditions [3].

Delonix regia (Hook) Raf is a species of small attractive tropical trees, it is commonly occurring flowering plant grown as an ornamental tree and given the name, flamboyant or flame tree, Gulmohar, peacock, Royal Poinciana [4]. Up till now eleven species were discovered in this genus, one occurs in Northeast Africa, nine species were found in endemic to Madagascar and the remaining species occurs from East and Northeast Africa to India. Chemical constituents of different classes such as; flavonoid, terpenoids and its glycosides, phenolics, phytosterol [5], were reported from flowers and leaves of *Delonix regia* (Hook) Raf species. The flower of *Delonix regia* (Hook) Raf was used as natural color and as an acid-base indicator. A number of published papers report the medicinal

properties for *Delonix regia* (Hook) Raf. The leaves are reported for its antimicrobial and antioxidant effect [6].

Body has itself antioxidant system, which reacts with reactive species and neutralizes them. This natural antioxidant system includes enzymes like catalase, superoxide dismutase and glutathione, which protect the body from free radical species and prevent oxidative stress [7]. Synthetic antioxidant like butylated hydroxyl toluene and butylated hydroxyl anisole are carcinogenic in nature. So, there arises a need for natural antioxidant [8]. With this background, the aim of the present study was to determine the possible phytochemical and antioxidant activity of chloroform extract of leaves.

MATERIALS AND METHODS

Plant Material

Plants were collected from Thanjavur District of Tamilnadu. The botanical identity of the plant of *Delonix regia* was confirmed by Dr. John Brito, Rapinet Herbarium, St. Joseph's College, Thiruchirappalli.

Preparation of Extract

The powder (8 kg) was extracted with 95% ethanol at room temperature for seven days. The extracts were filtered and concentrated under reduced pressure in a rotary evaporator and for further fractionated successively with n-hexane, chloroform, ethyl acetate, acetone, ethanol, butanol and methanol solvents were then removed under reduced pressure. From the fractionation chloroform extract were subjected to screening of phytochemical and antioxidant activity.

Phyto Chemical Analysis

Phytochemical analysis involves the qualitative analysis of herbal plants. The preliminary qualitative tests have been attempted in *Delonix regia* to find out the presence or absence of certain bio active Compounds. Chemical tests were carried out on the chloroform extract using standard procedures to identify the constituents as described by Harbone [9].

Test for Tannins

About 0.5g of the dried powdered samples was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added. Presence of brownish color indicating the Presence of tannins.

Test for Phlobatannins

Extract boiled with 1% aqueous hydrochloric acid. No red precipitate is formed. absence of phlobatannins.

Test for Saponin

About 2g of the powdered sample was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. No change in solution. Absence of Saponin.

Test for Flavonoids

Few drops of 1% aluminum solution were added to a portion of extract. A yellow colouration was observed indicating the Presence of flavonoids.

Test for Oil and Fat

Stain test:

Small Quantity of extract was pressed between two filter paper. An oily stain on filter paper indicates the presence of tilxed oil.

Test for Steroids

2ml of acetic anhydride was added to 0.5g ethanolic extract with 2ml sulphuric acid. The color changed from violet to blue indicating the Presence of steroids.

Test for Terpenoids (Salkowski Test)

5ml of extract was mixed in 2ml of chloroform, and 3ml of conc. Sulphuric acid was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids.

Test for Alkaloid

Mayer Test

0.5 ml of extract is added with 1 ml of mayer reagent. Yellow colour is absent indicating Absence of alkaloid.

Dragandroff's test

0.5ml of extract is added with 1ml of dragandroff's reagent orange red colour is absent Absence of Alkaloid.

Wagner Test

0.5ml Sample is treated with 1 ml of wagner reagent. No change in colour. Absence of alkaloids.

ANTIOXIDANT ACTIVITY

DPPH method

0.1 ml of the chloroform extract was taken in test tubes. 6 ml of DPPH (diphenyl picryl hydrazyl) solution was added and the tubes kept in dark for one hour. The color was read at 517 nm. The difference in the Optical density of DPPH solution and DPPH solution + sample was calculated. The decrease in OD with sample addition is used for calculation of the antioxidant activity. The activity was compared with BHT (butylated hydroxyl toluene) standard [10]. Free radical scavenging activity was expressed as the inhibition percentage calculated using the formula.

$$\text{Percentage of antioxidant activity} = \frac{[\text{Absorbance of control} - \text{Absorbance of sample}]}{\text{Absorbance of sample}} \times 100. \quad \text{-----Equation-1}$$

Reducing power assay

Reducing power assay of sample was done using published protocols [11]. 1 ml of chloroform extract was mixed with phosphate buffer (2.4 ml 0.2 M pH 6.6) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50 °C for 20 minutes. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and Ferricchloride (0.5 ml, 0.1%) and absorbance measured at 700 nm. Increased absorbance of the reaction mixture indicates stronger reducing power. The activity was compared with BHT standard. Scavenging activity was expressed as the

inhibition percentage calculated using the formula using equation 1.

Hydrogen peroxide scavenging activity

To determine the Hydrogen peroxide assay of chloroform extract by Umamaheswari and Chatterjee Method [12]. Hydrogen peroxide solution (2 mM/L) was prepared with standard phosphate buffer (pH- 7.4). Different concentration of the extracts in distilled water was added to 0.6 ml of hydrogen peroxide solution. Absorbance was determined at 230 nm after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The inhibition was calculated. BHT was used as standard. scavenging

activity was expressed as the inhibition percentage calculated using the formula using equation 1..

RESULTS AND DISCUSSION

The result of the phytochemical screening and antioxidant activity are given in the Table1, 2.

Terpenoids, fatty acids, flavonoids, steroids, Tannins were present in Delonix regia leaves and alkaloids, saponins, Pholobtannin were absent in chloroform extract of Delonix regia leaves.

Table 1: Qualitative Analysis of phytochemicals of Delonix regia leaves.

S.NO	PHYTOCHEMICAL	RESULT
1	Alkaloids	Absent
2	Pholobtannin	Absent
3	Tannin	Present
4	Terpenoid	Present
5	Saponin	Absent
6	Flavonoids	Present
7	Steroids	Present
8	Fatty acid	Present

Table 2: Antioxidant activity of chloroform extract of Delonix regia leaves.

S.NO	Method	Percentage of Antioxidant activity	Standard (BHT) (0.01 mg/ml)
1	DPPH assay	45.28	78.74
2	Reducing power assay	33.83	69.72
3	Hydrogen Peroxide assay	19.36	45.92

The antioxidant activity showed the inhibition percentage is 45.28 %, 33.83 % and 19.36 % respectively. The results observed from chloroform extract of Delonix regia leaves shows higher antioxidant potential and compared to three methods, DPPH assay observed high antioxidant activity. The results were compared to the standard BHT, it was only slight difference has been noted. The WHO estimated that 80% of the population of developing countries still relies on traditional medicine, mostly plant drugs for their primary health care needs. Hence, there is an urgent need to study the screening of antioxidant properties of herbs which will be helpful in the treatment of several diseases [13].

Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. Antioxidants may be synthetic or natural. Synthetic antioxidants such as BHT and BHA have recently been reported to be dangerous for human health. Thus, the search for effective, non-toxic natural compound with anti oxidative activity has been intensified in recent years [14]. On the basis of our results, delonix regia

appears to have potential for treatment of oxidative stress related diseases. It should, however, be explored as a functional medicinal plant for isolating the active ingredients along with animal studies in vivo.

ACKNOWLEDGEMENT

I wish to express my deep sense of gratitude and most sincere thanks to S. Kumaravel, senior scientist, Indian Institute of Crop Processing Technology, Thanjavur for providing all the facilities and support to finish my work.

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