

Phytochemical Constituents and Antioxidant Activity of *Delonix elata* L. in Flower Extract

Abstract

The objective of the present study was to evaluate the phytochemical constituents, we need to omit either the phytochemical or the total phenol and total because the one explain the other simultaneously. In addition, since there is nothing mentioned specifically about the phenol and flavonoid in the summary it is better only to use total phenol, and total flavonoids, anti oxidant activity of flower extract of *Delonix elata* have the maximum 93.1 % and 89.3 % antioxidant activity respectively, followed by chloroform extract of *delonix elata* ,89.3% antioxidant activity. Plants are widely used in pharmaceutical and food industries due to their biological importance. Among the plant parts, leaves, stem, roots and bark are widely studied for their biological properties. However, flowers are almost neglected and are not much probed for their importance. The present study was carried out to identify the phytochemicals and evaluate antioxidant activity of flowers of *Delonix elata*. The antioxidant activity was determined by the method of DPPH radical scavenging assay. The flower extract contain saponin, alkaloid, terpenoids, flavonoids, steroids, phenols, cardio glycosides, quinine coumarins and Tannins. Thus, clearly indicate that the flower extract of *Delonix elata* shows significant antioxidant activity which in turn greatly contribute in reducing the risk of many disease including ,heart disease, cancer cell formation and cell physiological abnormalities.

Keywords: Antioxidant activity; Flavonoids; Terpenoids; Tannins; *Delonix elata*

Research Article

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Introduction

In Ethiopia in the country sides where modern health care is not available, traditional medicine composite from different parts of plant are prescribed by the local physician to a patient to treat various critical disease. However only the local doctors have the knowledge of which medicinal plant has a therapeutic value to cure a specific disease. Nevertheless, the cognizance on the enormous existence of medicinal plants was very limited recently the awareness toward the importance of medicinal plants to treat various diseases and facilitate a number physiological activity in human body has shown a substantial progress. Generally, drugs synthesized from herbal medicinal plants are easily available, safe, less expensive and efficient. These drugs also have seldom side effects to the patient. According to World Health Organization, medicinal plants are the best source for the production of drugs [1]. Medicinal plants contain medicinally important bioactive phytochemicals include tannins, alkaloids, carbohydrates, terpenoids, steroids, flavonoids and phenols are synthesized by primary or rather secondary metabolism of living organisms. These organic compounds are primary metabolites and Secondary metabolites. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific and clinical researches [2]. Medicinal plant containing active chemical constituents with high antioxidant property has also an important role in the prevention of various degenerative diseases [3].

Delonix elata is commonly known as white gulmohur belonging to the family of *Fabaceae* and subfamily *Caesalpinioideae*. *Delonix elata* is not a classical Ayurvedic drug [4], but found includ-

ed in Shodala Nighantu under the Sanskrit name of Siddeshwara during 12 century AD [5]. The medicinal value of 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 [6,7]. Eventhough 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, they were inconsiderate of the flower part of the plant for its therapeutic value. The benefits may be attributed to the chemical constituents like β -sitosterol, quercetin, lupelol, lysine, alanine, valine, tyrosine and Rhamnose which are reported from *Delonix regia*. Quercetin 3-O-rhamnoglucoside and Quercetin-3-O-galactoside are also reported [8]. Extensive pharmacological studies on *Delonix elata* leaves and vegetative parts exhibited anti-inflammatory [7,9-11] anti-arthritic [7,9], immune modifying potentials and anti-oxidant activities [10] were studied. Hence, the present study was performed based on the phytochemical screening, total phenol, flavonoids, antioxidant activity, of flower extract of *Delonix elata*.

Materials and Methods

Preliminary phytochemical screening

The phytochemical composition of flower extract of *Delonix elata* L using commonly employed precipitation and coloration to investigate the presence of the major natural chemical groups such as steroids, alkaloids, phenolic compounds, Saponins, tannins, flavonoids, and cardio glycosides, quinone, terpenoid and coumarin, were performed by the standard method. Fresh flowers of *Delonix elata* were collected from different places of Chen-

nai. The flowers were washed thoroughly with normal tap water followed by sterile distilled water. Then the flowers were shade dried at room temperature, and turned into a fine powder after well grounding using grinding machine. Of the powdered flower, crude extract was prepared. Solvent used were ethanol, acetone, chloroform and petroleum ether, then each 1ml of extract was taken for the analysis of its phytochemical constituent.

Preparation of extracts

Preparation of the extracts was following the standard methods [12,13] About 15g of fine dried powdered flower of *Delonix elata* were mixed with 150mL of ethanol (75%), acetone, chloroform, petroleum ether aqueous using an Ultra Turax mixer for 1 min and soaked overnight at room temperature. The samples were then filtered through Whatman No.1 paper in Buchner funnel. The filtered solution was kept in a rotavator at 40 °C, then the dried powder filtrate of the flower extract of *Delonix elata* dissolved using different solvent was stored inside a freezer below 10 °C for the analysis.

Preliminary phytochemical analysis

Test for tannin: 1mL of 5% ferric chloride was added to 1mL of flower extract in a test tube, then the Formation of greenish black colour was taken as indicators for the presence of tannin.

Test for saponin: 2mL of distilled water was added to 1mL of flower extract in a test tube, then after the solution was shaken for 15minutes the formation of about 0.5 to 1cm layer of stable mass of bubbles observed as an indication for the presence of saponin.

Test for flavonoid: 1mL of 2N NaOH was added to 1mL of flower extract, than the result of yellow colour was taken as indicator for the presence of flavonoids.

Test for quinone: To 1mL of flower extract, 1.5mL of conc. sulfuric acid was added, than the solution was observed for the formation of red colour which indicates the presence of Quinone.

Test for cardio glycoside (kellerkilani test): To 1mL of flower extract, 2mL of glacial acetic acid and 0.5mL of 5% ferric chloride was added, then 1.5mL of concentrated sulfuric acid was added and observed for the formation of brown colour.

Test for terpenoid (salkowski test): 1mL of chloroform was added to 1mL of flower extract and 1.5mL of concentrated sulfuric acid is added to it. Formation of reddish brown colour indicates the presence of Terpenoids.

Test for phenol: To 1mL of flowers 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: 1mL of 10% Sodium hydroxide was added to 1mL of flower extract, than the solution was observed for the appearance of yellow colour.

Test for steroids: To 1mL of flower extract was added to 1mL of chloroform and 1.5mL of concentrated sulfuric acid. The appearance, at the inter phase, a reddish brown colour showed a positive reaction.

Test for alkaloid: To 1mL of flower extract, 1mL of concentrated sulfuric 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.

Estimation of total phenol content in flower extracts of *Delonix elata*

Total phenolic content in the flower extracts was estimated by the Folin-Ciocalteu colorimetric method as described by Maria et al. [14]. For the analysis, 0.5mL of diluted sample extract was added to 0.5mL of Folin-Ciocalteu reagent (0.5 N) and mixed thoroughly in the flask. Later 2.5 mL of sodium carbonate (2%) was added, and kept was for 30minutes. The absorbance was measured at 760 nm in a UV-Visible Spectrophotometer, then the total phenolic content of the extract were estimated using mg Gallic acid equivalents (GAE)/g.

Estimation of Total Flavonoid Content in Flower Extracts of *Delonix elata*

Total flavonoids content of flower extract of *Delonix elata* was determined by the aluminum chloride colorimetric method as described by Suriyavathana & Sivanarayan [15]. 0.5 mL of flower extracts of *Delonix elata* at a concentration of 1mg/ mL were taken and separately mixed with 3mL of methanol, 0.1mL AlCl₃ (10%), 0.1mL of potassium acetate and 2.8 mL distilled water. The solution was kept for 30 minutes and absorbance at 415 nm was recorded using UV/visible spectrophotometer. A standard calibration plot was generated at 415nm using quercetin solution at concentration equivalent/g of sample in methanol.

Qualitative Analysis of Antioxidant activity of *Delonix elata*

The antioxidant activity of flower extracts of *Delonix elata* was determined by using DPPH method Hsiao et al. [16]. 50µL of flower extracts of *Delonix elata* were taken in the microtiter plate. 100 µL of 0.1% methanolic 1,1-diphenyl-2-picrylhydrazyl (DPPH) was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration, from purple to yellow and pale pink were considered to be strong and weak positive respectively. The antioxidant positive samples were subjected for further quantitative analysis.

Quantitative Analysis of Free Radical scavenging activity of *Delonix elata*

The antioxidant activities were determined using DPPH, (Sigma-Aldrich) as a free radical as described by Babu et al. [17]. Flowers extract of 100µL were mixed with 2.7 mL of methanol and then 200µL of 0.1% methanolic DPPH was added. The suspension was incubated for 30minutes in dark condition. Initially, absorption of blank containing the same amount of methanol and DPPH solution was prepared and measured as a control [18]. Subsequently, at every 5 minutes interval, the absorption maxima of the solution were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of (0.16%) of Butylated hydroxy toluene (BHT). The experiment was carried out in triplicates.

The capacity of scavenging free radicals was calculated as scavenging activity (%) =

$$\frac{(Absorbance\ of\ control) - (Absorbance\ of\ sample)}{Absorbance\ of\ control} \times 100$$

Discussion

The Phytochemical analysis of flower extracts of *D. elata* demonstrated that among five different extracts of *D. elata* large amount of flavonoids, phenolic compounds, tannins, cardio glycosides, terpenoids, Quinone and Coumarin have found in the ethanolic flower extract of *D. elata*. The study also illustrated that of all extract of *D. elata*, ethanolic flower extracts were found rich in all tested phytochemical constituent with similar constituent analysis which has revealed from ethanolic leaf extracts Babu et al. [17]. However, ethanolic flower extract of *D. elata* contain abundant (+++) secondary metabolites than ethanolic leaf extract of *D. elata* including terpenoids, steroids, cardioglycoside, and quinine (Table 1). Since natural antioxidants which are found in plants are mainly in the form of phenolic compounds, such as flavonoids, phenolic acids, tocopherols [19]. The reported great free radical scavenging activity of flower extracts of *Delonix elata* perhaps is also due to liberal amount of flavonoids and phenolic components. These phyto compounds shows anti oxidative property in several physiological activity of living system by scavenging of free radicals, chelation of metal ions, such as iron and copper and inhibition of enzymes responsible for free radical generation [20]. In the present study, the most abundant extraction of antioxidants from flower of *Delonix elata* provided by ethanolic flower extract though 91.3% scavenging activity of flower extracts exhibited from acetone. These high scavenging activity implies that flower extracts of *Delonix elata* offer abundant antioxidants than ethanolic, aqueous, acetone, petroleum, ether and chloroform leaf extract of *D. elata* which is 74.01%, 69.29%, 51.96%, 57.48% and 4.45% respectively [17].

The therapeutic properties of medicinal plants are may be the result of the presence of various secondary metabolites such as alkaloids, flavonoids, cardio glycosides, phenols, saponins, steroids, etc [21]. Thus, the preliminary screening test is mandatory

to determine the bioactive principles which subsequently leads to the discovery and development of drugs [22]. The presence of alkaloids and saponins in the flower extract, the biological function of alkaloids and their derivatives are very important and are used in analgesic, antispasmodic and bactericidal activities [23]. Saponins have properties of precipitating and coagulating red blood cells, and they also have cholesterol binding properties, formation of foams in aqueous solutions and hemolytic activity [24], and traditionally Saponins have been extensively used as detergents and molluscicide, in addition to their industrial applications as foaming and surface active agents they also have beneficial health effects [25]. Plant steroids are known in facilitating cardio tonic activities and used in nutrition, cosmetics and herbal medicine.

Result revealed that *Delonix elata* flower consists of many useful compounds, such as flavonoids, tannins, phenols, saponins and alkaloids. Its antioxidant activity is largely due to flavonoids. The results further supported the view that the flowers of *Delonix elata* are promising source of naturally useful therapeutic agents. The estimation of total phenol and flavonoids content in the ethanolic extract of flower of *Delonix elata* as shown in (Table 2), the flower extract of *D. elata* contain high level of phenol and flavonoid compound which is 19mg GAE/g and 12.5mg GAE/g respectively. Phenolic compounds are important plant antioxidants which exhibited considerable scavenging activity against radicals [26]. Therefore, the antioxidant activity of sample extracts can be attributed mainly of its phenolic compounds [27-29]. Similarly, Shahidi & Naczek [30] reported that naturally occurring phenolic exhibit antioxidant activity. *Delonix elata* flower extracts were further analyzed for the presence of antioxidants. The results revealed strong positive response for acetone flower extract followed by others. Scavenging activity for free radicals of DPPH has been widely used to evaluate the antioxidant activity of natural products from plant and natural sources (Table 3). Free radicals have a broad range of effects in biological systems. It has been proved that these mechanisms may be important in the pathogenesis of certain diseases and ageing. Many synthetic antioxidant components have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidant [31-75].

Table 1: Phytochemical screening of Flowers of *Delonix elata*.

| Phytochemicals | Aqueous Extract | Ethanolic Extract | Chloroform Extract | Acetone Extract | Petroleum ether Extract |
|------------------|-----------------|-------------------|--------------------|-----------------|-------------------------|
| Tannin | ++ | +++ | - | +++ | - |
| Saponin | ++ | +++ | - | - | - |
| Flavonoid | + | ++ | + | + | + |
| Quinone | + | +++ | + | ++ | - |
| Cardio Glycoside | + | ++ | + | ++ | + |
| Terpenoid | + | ++ | + | ++ | + |
| Phenol | ++ | +++ | + | ++ | + |
| Coumarin | + | ++ | + | + | + |
| Steroid | + | ++ | + | ++ | + |
| Alkaloid | + | + | - | + | - |

Table 2: Estimation of total phenol and flavonoid content of ethanolic flower extract of *Delonix elata*. GAE: Gallic Acid Equivalent.

| Plant Name | Total Phenol Content (mg GAE/g) | Total Flavonoid Content (mg /g) |
|----------------------|---------------------------------|---------------------------------|
| <i>Delonix elata</i> | 19 | 12.5 |

Table 3: DPPH scavenging activity (in %) of flower extract of *Delonix elata*.

| Flower Extracts of <i>D. elata</i> | % of Inhibition for 100 µl |
|------------------------------------|----------------------------|
| Petroleum Ether | 81.3 |
| Chloroform | 88.6 |
| Acetone | 91.3 |
| Ethanol | 86 |
| Aqueous | 89.3 |
| BHT (Standard) | 98.6 |

Conclusion

The present study revealed that flower extract of *Delonix elata* was rich in phytochemical constituents and high levels of total phenolic and flavonoids compounds. The flower extract of *Delonix elata* also possessed strong antioxidant potential and was thus capable of inhibiting, quenching free radicals to terminate the radical chain reaction. The results indicate that the plant material may become an important source of natural drug compounds with health protective potential and natural antioxidants of significant impact on the status of human health. Therefore, traditional medicine practice is recommended strongly for this plant and further study should be carried out to isolate, purify, and characterize the active constituents responsible for the bioactivity study and disease prevention.

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