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STUDIES ON PHYTOCHEMICAL CONSTITUENTS AND ANTIOXIDANT ACTIVITY OF *Delonix elata* L. FLOWER

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ABSTRACT

The objective of the present study was to evaluate the phytochemical constituents, total phenol, total flavonoid, anti-oxidant activity of flower extract of *Delonix elata.* 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 *D.elata*. The antioxidant activity was determined by the method of DPPH radical scavenging assay. The flower extract contain saponin, alkaloid, terpenoids, flavonoids, steroids, phenols, cardioglycosides, quinones coumarins and Tannins. Thus the in-vitro studies clearly indicate that the flower extract of *D.elata* shows significant antioxidant activity which would be helpful in prevention of various oxidative stresses.

KEYWORDS: Antioxidant activity, flavonoids, terpenoids, Tannins, *Delonix elata.*

INTRODUCTION

In the ancient India, medicinal plants were used to prevent various critical diseases. Even in recent years, there has been an increasing awareness about the importance of medicinal plants. Generally, herbal drugs are easily available, safe, less expensive, efficient, and rarely have side effects. According to World Health Organization, medicinal plants would be the best source to obtain variety of drugs.[1]

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids,

carbohydrates, terpenoids, steroids, flavonoids and phenols. The bio-active phytocompounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas.[2] Medicinal plants containing active chemical constituents with high antioxidant property play an important role in the prevention of various degenerative diseases[3] and have possible benefits to the humanity.

Delonix elata is commonly known as white gulmohur belonging to the family of *Fabaceae* and subfamily *Caesalpinoidae*. *Delonix elata* is not a classical Ayurvedic drug,[4] but found included in Shodhala Nighantu under the Sanskrit name of '-Siddeshwara-' during 12century AD.[5] 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.[6,7] 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 which are reported from *Delonix regia.* Quercetin 3-O-rhamnoglucoside and Quercetin-3O-galactoside are also reported.[13] Extensive pharmacological studies on *D.elata* leaves and vegetative parts exhibited anti-inflammatory[7,8,9,10] anti- arthritic,[7,8] immune modifying potentials and anti-oxidant activities[11] were studied. Hence, the present study was performed to focused on the phytochemical screening, total phenol, flavonoid, antioxidant activity, of flower extract of *D.elata*.

MATERIALS AND METHODS

PRELIMINARY PHYTOCHEMICAL SCREENING

The phytochemical qualitative chemical composition of flower extract of *Delonix elata* L. using commonly employed precipitation and coloration to identify the major natural chemical groups such as steroids, alkaloids, phenolic compounds, saponins, tannins, flavonoids, and cardioglycosides were performed by the standard method. General reactions in the analysis revealed the presence or absence of these compounds in the crude extracts tested.

Fresh flowers of *D.elata* were collected from different places of chennai. The flowers were washed thoroughly with normal tap water followed by sterile distilled water. Then the

flowers were shade dried at room temperature. These were crushed to powder using grinding machine.

The powered sample was analysed for qualitative inorganic compounds.

PREPARATION OF EXTRACTS

Preparation of the extracts was following the standard methods.[12,13] About 15g of fine dried powdered flowers of *D.elata* were extracted with 150mL ethanol (75%), acetone, chloroform, petroleum ether aqueous extract for 1 min using an Ultra Turax mixer (13,000rpm) and soaked overnight at room temperature. The samples were 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 solvent. The concentrated extracts were stored in airtight container in refrigerator below 10°C.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Test for Tannin

1 mL of flower 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 flower 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 flower extract. Appearance of yellow colour indicates the presence of flavonoid.

Test for Quinone

To 1mL of flower 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 flower 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 flower 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 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

Add 1mL of 10% Sodium hydroxide to 1mL of flower extract. 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 conc.sulphuric acid. The appearance, at the interphase,a reddish brown colour showed a positive reaction.

Test for Alkaloid

To 1mL of flower 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.

ESTIMATION OF TOTAL PHENOL CONTENT IN FLOWER EXTRACTS OF *D.ELATA*

Total phenolic content in the flower extracts was determined by the Folin–Ciocalteau colorimetric method.[14] For the analysis, 0.5 mL of aliquot of sample was added to 0.5 mL of Folin–Ciocalteau reagent (0.5 N) and the contents of the flask were mixed thoroughly. Later 2.5 mL of sodium carbonate (2%) was added, and the mixture was allowed to stand for 30 minutes after mixing. The absorbance was measured at 760 nm in a UV-Visible Spectrophotometer. The total phenolics contents were expressed as mg gallic acid equivalents (GAE)/g extract.

ESTIMATION OF TOTAL FLAVONOID CONTENT IN FLOWER EXTRACTS OF *D.ELATA*

Total flavonoids content of flower extract of *D.elata* was determined by the aluminium chloride colorimetric method.[15] 0.5 mL of flower extracts of *D.elata* at a concentration of 1mg/ mL were taken and the volume was made up to 3mL with methanol. Then 0.1mL $AlCl_3$

(10%), 0.1mL of potassium acetate and 2.8 mL distilled water were added sequentially. The test solution was vigorously shaken. Absorbance was recorded at 415 nm after 30 minutes of incubation. A standard calibration plot was generated at 415nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent /g of sample.

QUALITATIVE ANALYSIS OF ANTIOXIDANT ACTIVITY OF *D.ELATA*

The antioxidant activity of flower extracts of *D.elata* was determined by standard method.[16,17] 50 µL of flower extracts of *D.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 *D.ELATA*

The antioxidant activities were determined using DPPH, (Sigma-Aldrich) as a free radical. Flowers extract of 100 µL were mixed with 2.7 mL of methanol and then 200 µL of 0.1% methanolic DPPH was added. The suspension was incubated for 30 minutes 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 517 nm. 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 (%) =

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

RESULT AND DISCUSSION

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	+	+++	+	++	-
Cardioglycoside	+	++	+	++	+
Terpenoid	+	++	+	++	+
Phenol	++	+++	+	++	+
Coumarin	+	++	+	+	+
Steroid	+	++	+	++	+
Alkaloid	+	+	-	+	-

(+) *Positive* (++) Strong *Positive* (-) *Negative*

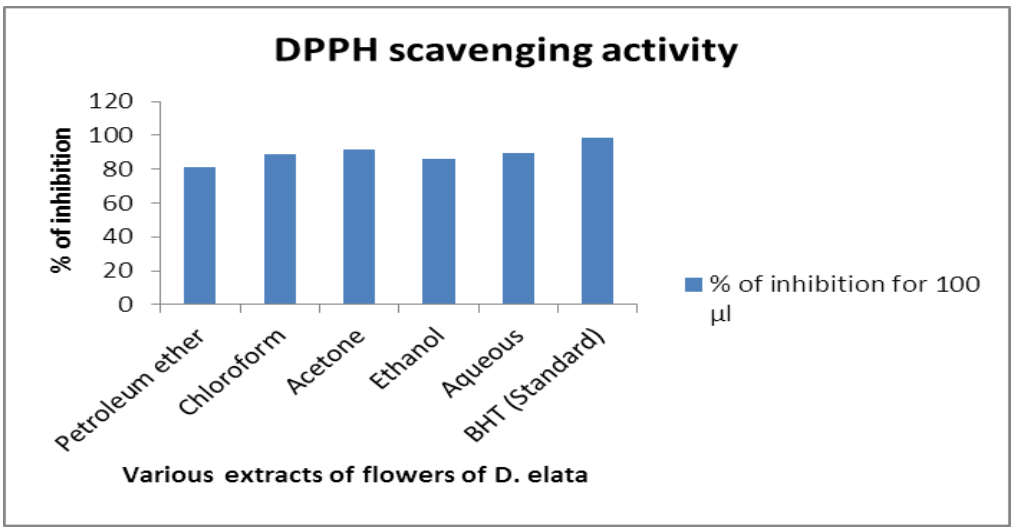
Table 2: Estimation of total phenol and flavonoid content of ethanolic flower extract of *Delonix elata*

Plant name	Total phenol content (mg GAE/g)	Total flavonoid content (mg /g)
Delonix elata	19	12.5

GAE: Gallic acid equivalent

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

Flower extracts of *D. elata*	% of inhibition for 100 µl
Petroleum ether	81.3
Chloroform	88.6
Acetone	91.3
Ethanol	86
Aqueous	89.3
BHT (Standard)	98.6



DISCUSSION

Medicinal plants are of great importance to the health of individuals and communities [19]. Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities [20]. Analysis of the plant extracts revealed the presence of phytochemicals, such as, tannins, saponin, phenols, tannins, flavonoids, coumarins, quinone, cardioglycosides, steroids, terpenoids and alkaloids. Several studies have described the antioxidant properties of different parts of various medicinal plants which are rich in phenolic compounds.[21,22] *D. elata* is a widespread medicinal plant used in the pharmacological system of medicine to care for various degenerative diseases. Phytochemical analysis revealed a large amount of flavonoids, phenolic compounds and tannins and cardioglycosides, terpenoid, quinone and coumarin. Natural antioxidants mainly come from plants in the form of phenolic compounds, such as flavonoids, phenolic acids, tocopherols etc.[23] The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper and inhibition of enzymes responsible for free radical generation.[24] This flower extract has great free radical scavenging property and also contains liberal amount of flavonoid and phenolic components. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms in vitro.

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, cardioglycosides, phenols, saponins, steroids, etc.,.[25] Thus, the preliminary screening test may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development [26]. 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.[27] 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[28] and traditionally saponins have been extensively used as detergents and molluscicides, in addition to their industrial applications as foaming and surface active agents and also have beneficial health effects.[29] Plant steroids are known important for their cardiotonic activities and also used in nutrition, herbal medicine and cosmetics.

Result revealed that *D. 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 *D.elata* are promising source of natural useful therapeutic agents. The traditional medicine practice is recommended strongly for this plant as well as it is suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the bioactivity study.

The estimation of total phenol and flavonoid content in the ethanolic extract of flower of *D.elata* showed in Table 2. Phenolic compounds are important plant antioxidants which exhibited considerable scavenging activity against radicals. It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers.[30] Thus, antioxidant capacity of a sample can be attributed mainly to its phenolic compounds.[31-33] Similarly, Shahidi and Naczk[34] reported that naturally occurring phenolics exhibit antioxidant activity.

D.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. 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.[35-37]

CONCLUSION

The present study revealed that flower extract of *D.elata*a was rich in phytochemical constituents and high levels of total phenolic and flavonoid compounds. The flower extract of *D.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 and disease prevention.

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