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SCREENING OF PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY OF LEAF EXTRACTS OF DELONIX ELATA L.

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ABSTRACT

Antioxidants are inhibitors in 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 the antioxidant activity of Delonix elata leaves. The antioxidant activity was determined by the method of DPPH radical scavenging assay. The ethanolic extract of the leaves contain terpenoids, flavonoids, steroids, phenols, cardioglycosides, quinines, coumarins and tannins. Thus the in vitro study clearly indicates that the ethanolicc extract of D.elata leaves showed 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, tannins, Delonix elata.

INTRODUCTION

Medicinal plants typically contain mixtures of different chemical compounds that may act individually, additively or in synergy to improve health. The main objective of this study is, to search the plant with strong antioxidant activity which could serve as good candidate for the development of standardized phytomedicines. Free radicals contribute to more than one hundred disorders in human beings including atherosclerosis, arthritis, ischemia, central nervous system injury, gastritis, cancer and AIDS.[1] Antioxidants can be effective in preventing free radical formation by scavenging them or increasing their decomposition rate and suppressing such disorders.[2] Currently, there is a growing interest toward natural antioxidants of herbal resources.

Currently, the use of antioxidative phytochemicals such as polyphenols, vitamin C, phenolic acids and flavonoids in food is gaining popularity due to their anticarcinogenic activity, potential health benefits including the prevention and lowering risk of development of cancer, heart and neurodegenerative disorders.[1-8] Several natural antioxidants are believed to play an important role in ameliorating oxidation process by quenching free radicals, chelating catalytic metals and scavenging oxygen in foods and biological systems.[9-10]

Delonix elata is commonly known as white gulmohur belonging to the family Fabaceae and subfamily Caesalpinoidae. Delonix elata is not a classical Ayurvedic drug.[11] but found included in Shodhala Nighantu under the Sanskrit name of '-Siddeshwara-' during 12th century AD.[12] The medicinal use of the tree is acknowledged by people living in the villages who take decoction of the leaves and barks to get relief from rheumatic problems like pain and stiffness of the joints, especially affecting the knees.[13,14] It was observed that local people and Siddha practitioners in Tamil Nadu, India use 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 Delonix elata exhibited anti-inflammatory.[14-17] anti- arthritic.[14-15] immune modifying potentials and anti-oxidant activities.[18] were studied.

MATERIALS AND METHODS

Preparation of extracts

Preparation of the extracts was following the standard methods.[19-20] About 15g of fine dried powdered leaves of D.elata were extracted with 150mL of ethanol (75%), chloroform, petroleum ether and water. They were mixed 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 extract were stored in airtight container in refrigerator below 10°C.

Preliminary phytochemical screening

The qualitative phytochemical composition of ethanolic extract of Delonix elata L. was performed using commonly employed precipitation and coloration methods to identify the major natural chemical groups such as steroids, reducing sugars, alkaloids, phenolic

compounds, saponins, tannins, flavonoids, amino acids and glycosides. General reactions in these analysis revealed the presence or absence of these compounds in the crude extracts tested.

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 powered sample was analysed for qualitative inorganic compounds.

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 indicates 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 the presence of alkaloids.

QUALITATIVE ANTIOXIDANT ACTIVITY

Antioxidant assay of D.elata was estimated for its free radical scavenging activity by using DPPH (1,1-Diphenyl-2-Picrylhydrazyl) free radical assay.

DPPH is a stable free radical with purple colour (absorbed at 517nm). If free radicals have been scavenged, DPPH will degenerate to yellow colour. This assay uses this character to show free radical scavenging activity.

50µL of leaf extract of D.elata was taken in a micro-titre plate. 100µL of 0.1 % methanolic DPPH was added over the sample and incubated for 30 minutes in dark condition. The samples were then taken, observed for discolouration from purple to yellow and pale pink were considered as strong and weak positive respectively. The anti-oxidant positive samples were subjected for further quantitative analysis.

QUANTITATIVE ANTIOXIDANT ACTIVITY

Leaf extract samples of 100µL from qualitative assay were mixed with 2.7mL of methanol and then 200µL of 0.1% methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Subsequently at every 5 minutes intervals, the absorption maxima of the solution were measured using UV Double Beam Spectra Scan (Chemito, India) at 517nm. The anti-oxidant activity of the sample was compared with known synthetic standard of 0.16% of Butylated Hydroxy Toluene (BHT).

The free radical activity of the sample is calculated by the following formula

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

RESULTS

Table 1: Phytochemical screening of Leaves of Delonix elata

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

(+) Presence of phytochemicals (++) Strong presence of phytochemicals

(-) Absence of phytochemicals

Table 2. DPPH scavenging activity (in %) of Leaf extract of Delonix elata.

Time in mins	% of inhibition of 100 µl of different leaf extracts					
	Pet ether	Chloroform	Acetone	Ethanol	Aqueous	BHT
0	79.8	85.7	90.9	92.8	90.2	61.0
5	79.6	87.5	90.7	96.0	93.4	90.7
10	80.1	88.0	90.7	96.0	94.0	97.3
15	80.1	88.0	90.7	96.0	94.0	98.0
20	80.0	88.0	90.6	96.0	94.0	98.6
25	80.0	88.6	90.6	96.0	94.0	98.6
30	80.0	88.6	90.6	96.0	94.0	98.6

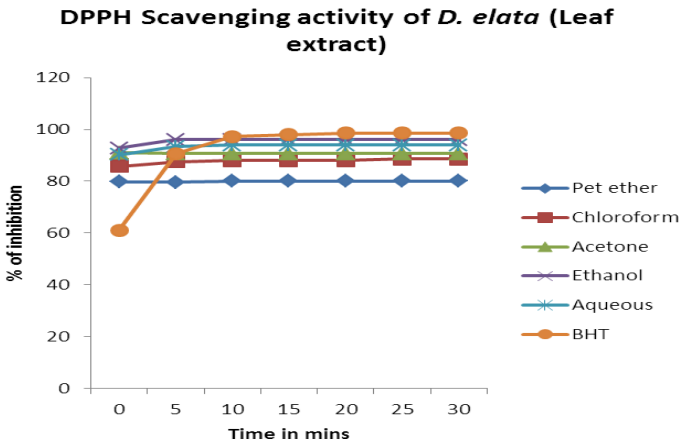

Fig. 1. DPPH scavenging activity (in %) of Leaf extract of Delonix elata

DISCUSSION

The preliminary phytochemical screening of leaf extracts of D.elata (Table 1) showed the presence and absence of alkaloids, saponins, steroids, flavonoids, phenolic compounds, tannins, quinone, cardioglycoside, terpenoid and coumarin in the ethanolic extract compared to other extracts.

The results showed predominant presence of tannins, flavonoids, cardioglycoside, terpenoid, phenols, steroids, alkaloids, quionones and coumarins in the plant D.elata. Hence the plant has undoubtedly contributed to its traditional medicinal value, and thus confirmed that it can serve as a potential source of useful drugs in the near future, if further studies are conducted.

The in vitro antioxidant activity of leaf extracts of D.elata (Table 2) the percentage of DPPH (free radicals) scavenging activity is increased with respect to the concentration of the plant extracts. Among the five solvents, ethanolic extract of D. elata shows notable DPPH scavenging activity.

The results observed from ethnolic extract of D. elata leaves shows higher antioxidant potential when compared to others solvents, DPPH assay observed high antioxidant activity. The results were compared to the standard BHT, where 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.[21] 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 harmful for human health. Thus, the search for effective, non-toxic natural compound with anti oxidative activity has been intensified in recent years.[22] On the basis of our results, D.elata appears to have potential for treatment of oxidative stress related diseases.

High phenolic compounds in ethanol extracts play the role of potent antioxidants and antimicrobial activity. Flavonoids have been proven to display a wide range of pharmacological and biochemical actions, such as antimicrobial and anticarcinogenic activities.[23] Flavonoids can act as free radical scavengers and terminate the radical chain reactions that occur during the oxidation of triglycerides in the food systems.[24] Moreover, the presence of tannins in the extracts may explain its potent bioactivities which are known to possess potent antioxidants.[25]

CONCLUSION

The results revealed the presence of substantial amount of bioactive constituents comprising cardioglycosides, terpenoids, phenols, flavonoids, steroids, alkaloids, quionones and coumarins in D.elata was observed comparatively richer source of these phytochemicals. The results provide evidence that the plant possess potent source of natural antioxidant and medicinally important compounds. The results also conclude that the antioxidant activity of alcoholic (ethanol) extract of D. elata is higher than that of other extracts. The notable quantity of phenols and flavonoids may be responsible for its higher antioxidant activity.

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