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Comparative Phytochemical Investigation and Determination of Total Phenols and Flavonoid Concentration in Leaves and Flowers Extract of *Delonix regia (Boj. Ex. Hook)*

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ABSTRACT

Medicinal plants are the potent source of biologically active compounds and have always been of field of interest for the effective chemotherapeutic agents and offering a broad spectrum of activity with greater emphasis on preventive action. The objective of the present study was to evaluate the phytochemical constituents, total phenol and total flavonoids content in leaves and flowers different extract of *Delonix regia.* Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by Folins Ciocalteau reagent method and aluminium chloride method respectively. Phytochemical analysis revealed the presence of phenols, flavonoids, alkaloids and carbohydrate. The present study concluded that the crude extract of *Delonix regia* is a rich source of secondary phytoconstituents which impart significant antioxidant potential. It is expected that the important phytochemical properties recognized by our study in the indigenous medicinal plants will be very useful in the curing of various diseases when taken along with our food.

Keywords: *Delonix regia*, Phytochemical screening, Folins ciocalteau reagent, Aluminium chloride

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INTRODUCTION

Medicinal plants are rich source of novel drugs that forms the ingredients in traditional system of medicine, modern medicines, pharmaceutical intermediates and lead compounds in synthetic drugs[1]. The reason for using them as medicine lies in the fact that they contain chemical components of therapeutic value[2]. These compounds are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. The medicinal value of plants lies in some chemical substances (usually secondary metabolites) that produce a definite physiological action as the human body. In recent times focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems[3] including treatment against hepatocellular carcinoma[4]. Herbal medicines are being used by nearly about 80% of the world population, primarily in developing countries for primary health care[5]. Assessing the current status of health care system, inadequacies of synthetic drugs are likely to be more glaring in the coming years. *Delonix regia* (Boj. Ex. Hook) (Family: Caesalpiniaceae) is a medium-sized tree found in greater parts of India. The decoction of the leaves is traditionally used in treating gastric problems, body pain and rheumatic pains of joints[6, 7]. Ethanolic extracts of flower and bark were investigated to anti-inflammatory activity in rats[8]. The leaves are reported to antibacterial[9] and antimalarial[10]. *Delonix regia* contains proteins, flavonoids, tannins, phenolic compounds, glycosides, sterols, and triterpenoids. The present study was designed to investigate the presence of various phytochemicals constituents in *Delonix regia* leaves and flowers. Extensive effort have now been challenged towards screening of plants for more active and effective new drugs to eliminate diseases which have strains of pathogenic organism that resist the effect of drug in use today[11]. Based on the many ethno medicinal values of this plant, it is becomes imperative to determine the active ingredients present in different parts of the plant as well as their composition.

MATERIALS AND METHODS

Plant material

The leaves and flowers of *Delonix regia were* collected from ruler area of Bhopal (M.P.) in the month of Feb, 2015. The plant sample were separated and washed with sterile distilled water to remove the adhering dust particles and other unwanted materials. The leaf and flowers was air dried

under room temperature. The dried plant samples were cut and grinded to make it in powder form. The powdered samples were stored in clean, dry and sterile container for further use.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Defatting of plant material

Powdered plants material of *Delonix regia* were shade dried at room temperature. The shade dried plants material was coarsely powdered and subjected to extraction with petroleum ether using soxhlation method. The extraction was continued till the defatting of the material had taken place.

Extraction by soxhlation process

Dried 42 gram of leaves and 45 gram of flower of *Delonix regia* were exhaustively extracted with different solvent using soxhlation method. The extracts were evaporated above their boiling points and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts[12].

Qualitative phytochemical analysis of plant extract

The *Delonix regia* extracts obtained was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate[13, 14]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, protein and amino acid and Diterpenes

Total phenol determination

The total phenolic content was determined using the method of Olufunmiso *et al*[15]. A volume of 2ml of each extracts or standard was mixed with 1 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The colour developed was read at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

Total flavonoids determination

The total flavonoid content was determined using the method of Olufunmiso *et al*[15]. 1ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

RESULTS AND DISCUSSIONS

The crude extracts so obtained after each of the successive soxhlation extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The yield of extracts obtained from the leaves of the plants using chloroform, ethyl acetate, ethanol and water as solvents are depicted in the Table 1.

Table 1 Result of percentage yield of extracts of *Delonix regia*

S. No.	Solvents	Percentage Yield (%)	
		Leaves	Flowers
1.	Pet ether	1.766	1.566
2.	Chloroform	2.116	8.815
3.	Ethyl acetate	1.111	1.917
4.	Ethanol	1.152	6.135
5.	Aqueous	6.869	30.30

The results of qualitative phytochemical analysis of the crude powder of leaf and flower of *Delonix regia* were shown in Table 2. Ethanolic and aqueous extracts of *Delonix regia* leaves showed the presence of alkaloids, flavonoids, saponins, phenols, and carbohydrate and flower extract shown the presence of alkaloids, flavonoids, saponins, and phenols.

Table 2 Result of phytochemical screening of extracts of *Delonix regia* (Leaves)

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
1.	Alkaloids				
	A) Wagner's Test:	+Ve	+Ve	+Ve	+Ve
	B) Hager's Test:	+Ve	+Ve	+Ve	+Ve
2.	Glycosides				
	A) Legal's Test:	-Ve	-Ve	-Ve	+Ve
3.	Flavonoids				
	A) Lead acetate Test:	-Ve	+Ve	+Ve	+Ve
	B) Alkaline Reagent Test:	-Ve	+Ve	+Ve	-Ve
4.	Saponins				
	A) Froth Test:	-Ve	-Ve	+Ve	+Ve
5.	Phenolics				
	A) Ferric Chloride Test:	-Ve	+Ve	+Ve	+Ve
6.	Proteins and Amino Acids				
	A) Xanthoproteic Test:	-Ve	+Ve	-Ve	+Ve
7.	Carbohydrate				
	A) Fehling's Test:	-Ve	-Ve	+Ve	+Ve
8.	Diterpenes				
	A) Copper acetate Test:	-Ve	+Ve	-Ve	+Ve

Table 3 Result of phytochemical screening of extracts of *Delonix regia* (Flowers)

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
1.	Alkaloids				
	A) Wagner's Test:	-Ve	-Ve	-Ve	+Ve
	B) Hager's Test:	-Ve	-Ve	-Ve	+Ve
2.	Glycosides				
	A) Legal's Test:	-Ve	-Ve	-Ve	+Ve
3.	Flavonoids				
	A) Lead acetate Test:	-Ve	+Ve	+Ve	-Ve
	B) Alkaline Reagent Test:	+Ve	+Ve	-Ve	+Ve
4.	Saponins				
	A) Froth Test:	-Ve	+Ve	+Ve	+Ve
5.	Phenolics				
	A) Ferric Chloride Test:	-Ve	+Ve	+Ve	+Ve
6.	Proteins and Amino Acids				
	A) Xanthoproteic Test:	-Ve	+Ve	+Ve	-Ve
7.	Carbohydrate				
	A) Fehling's Test:	-Ve	-Ve	-Ve	-Ve
8.	Diterpenes				
	A) Copper acetate Test:	-Ve	-Ve	-Ve	+Ve

The content of total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **Y = 0.042X+0.002, R^2= 0.999,** where X is the gallic acid equivalent (GAE) and Y is the absorbance. The content of total flavonoid compounds (TFC) was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: **Y = 0.06X+0.019, R^2= 0.999,** where X is the quercetin equivalent (QE) and Y is the absorbance. The total phenol and flavonoid content of *Delonix regia* leaf and flower extract was present Table 4 and 5 respectively.

Table 4 Total phenolic and total flavonoid content of *Delonix regia* (Leaves)

S. No.	Extracts	Total Phenol (GAE) (mg/100mg)	Total flavonoid (QE) (mg/100mg)
1.	Ethyl acetate extract	3.43	3.96
2.	Ethanol extract	3.56	1.03
3.	Aqueous extract	3.21	0.92

Table 5 Total phenolic and total flavonoid content of *Delonix regia* (Flowers)

S. No.	Extracts	Total Phenol (GAE) (mg/100mg)	Total flavonoid (QE) (mg/100mg)
1.	Chloroform extract	-	0.77
2.	Ethyl acetate extract	4.07	1.37
3.	Ethanol extract	3.42	1.22
4.	Aqueous extract	3.10	0.633

CONCLUSION

Qualitative and quantitative analysis of phenolics and flavonoids from leaves and flower extract of *Delonix regia* was achieved first time in this work. The observed level of phytoconstituents revealed that *Delonix regia* is a rich source of antioxidant compounds. Currently available synthetic antioxidants are suspected to cause or prompt negative health effects, hence strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Moreover, the plant parts may be used as an alternative source for flavonoids and phenols for traditional remedies. Further phytochemical studies are also required to isolate and characterize active ingredients that are responsible for its antioxidant and others activity and to explore the existence of synergism if any, among the compounds.

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