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INVIRTO ANTIOXIDANT PROPERTY OF *DELONIX REGIA*

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ABSTRACT

Delonix regia commonly called as Gulmohar, used as folk medicine. The present study is carried out using extract of flowers and bark of *Delonix regia* on lipid peroxidation using egg yolk method and carrying out TBARS assay to test the antioxidant activity in vitro.

Key words: Antioxidants, TBARS, Lipid peroxidation, *Delonix regia*.

INTRODUCTION

Natural source is potential for new drugs, many plants have phytochemical which act as antioxidant (Rudy J *et al.*, 2006). Evidence for many studies have proven the concept of free radical injury in various disorder especially neurodegenerative disorder Parkinsonism, cancer, diabetes, hypertension, rheumatoid arthritis etc (Dreher D and Junod AF, 1996; dhalla NS *et al.*, 2000; Marnett LJ, 2000; Niedowicz DM and Daleke DL, 2005; Saleh NAM, Ishak MS, 1976; Amit K and Priyadarsini KI, 2011). Antioxidant protect the cell from injury by scavenging free radical or either by increasing antioxidant defence or by directly inhibiting the pathway like lipid peroxidation. Antioxidant have been in market since many years there is ample of evidence of increasing utility of antioxidant product in the field of medicine (Zadak Z *et al.*, 2009). Also modern life is stressfull, stress can also increase free radical injury producing early morbidity and increases the risk of mortality. *Delonix regia* have flavonoids which have known antioxidant property (Adje F *et al.*, 2008; Joy PP *et al.*, 2001; Jyothi MV *et al.*, 2007; Sammour RH, El-Shanshoury AR, 1992).

Botanical general characters of family *Leguminosae*

The family *Leguminosae* includes trees, shrubs,

woody vines, and annual or perennial herbs. Leaves are usually alternate and compound–bipinnate, simple pinnate, or palmate, but rarely simple. Inflorescence is variously racemose, in simple racemes, panicles, spikes, or heads. Flower structure varies to the extent that 3 subfamilies (*Mimosoideae*, *Caesalpinioideae*, *Papilionoideae*) are recognized; corolla typically 5-parted; stamens 3-many, mostly 10, free, or united by their filaments in various ways, pistil simple, free. The Fruit is characteristically a legume, dehiscent or indehiscent.

Delonix regia

Delonix regia is a species of flowering plant from the *Fabaceae* family (the new name of the family *Leguminosae*). This tree is native to Madagascar and is often grown as an ornamental tree in many countries around the world, consistently voted among the Top Five most beautiful flowering trees in the world. *Delonix regia* is also known as the Royal Poinciana or Flamboyant (Al-Bahry S *et al.*, 2005). This plant was previously placed in the genus *Poinciana*, named after Phillippe de Longvilliers de Poincy (1583-1660), who is credited with introducing the plant to the Americas.

General botanical character of *Delonix regia*

The Royal Poinciana grows to a height of 9-15 meters with an elegant, wide-spreading, umbrella-like canopy which sometimes exceeds the height of the tree in diameter. Royal Poinciana is deciduous in climates that

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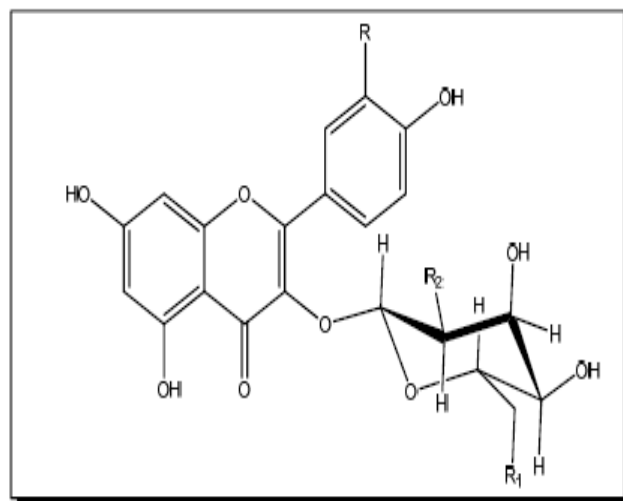
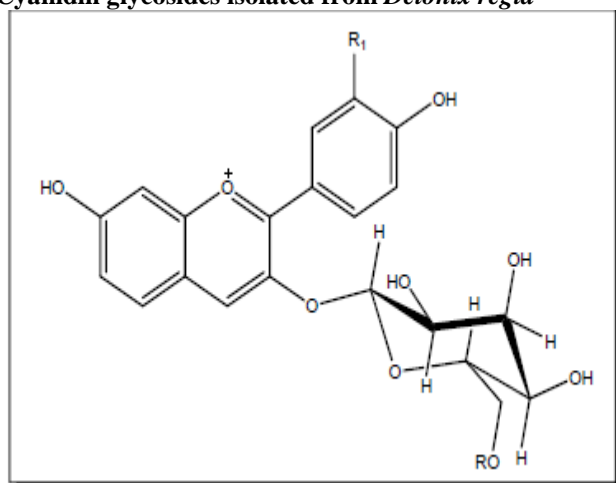
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have a marked dry season, but in tropical areas (where the winter is not that much dryer than the summer), it is a semi-evergreen tree.

The leaves are even-pinnate, alternate, and can grow up to 66 cm long, 10-25 pairs of opposite pinnate, and 12-40 pairs of small oblong leaflets. The flowers are Caesalpinaceae, can grow up to 12 cm wide, consisting of 5 red or yellow petals, or standard of red splashed with burgundy spots. They are arranged on racemes that can reach up to 25 cm long. The fruits are Pods, long, hard, flat, slightly curved and dehiscent (Gilman EF, Watson DG, 1993; Adje F *et al.*, 2008).

Many publications take in consideration the *Delonix regia* fatty acids and sterols (Stigmasterol, γ -Sitosterol, β -Amyrin) as well as protein and lectin content. In contrast only two of these publications deal with the isolation and characterization of *Delonix regia* flavonoids (Joy PP *et al.*, 2001). The flavonoids isolated from this plant are summarized in Table 1 and Table 2.

Cyanidin glycosides isolated from *Delonix regia*



Traditional uses of *Delonix regia*

The extract of *Delonix regia* is known to have medicinal properties. This plant is used in several countries to prepare extracts with antimicrobial and antifungal activities (Cao G *et al.*, 1997).

Antioxidant activities associated with flavonoids

Numerous publications have investigated the antioxidant activities of flavonoids and how they can contribute to the treatment of several diseases. Considering these publications, they indicate that biological and pharmacological effects of flavonoids may depend upon their behavior as either antioxidants or as prooxidants. Some flavonoids can behave as both antioxidants and prooxidants, depending on concentration and the redox environment present. For instance, certain flavonoids act as antioxidants against free radicals, yet demonstrate prooxidant activity when a transition metal such as Cu²⁺ is present (Wardman P, 1989).

Three important points must be illustrated in order to better understand antioxidant activity of flavonoids and the important role of flavonoids compounds in human biology:

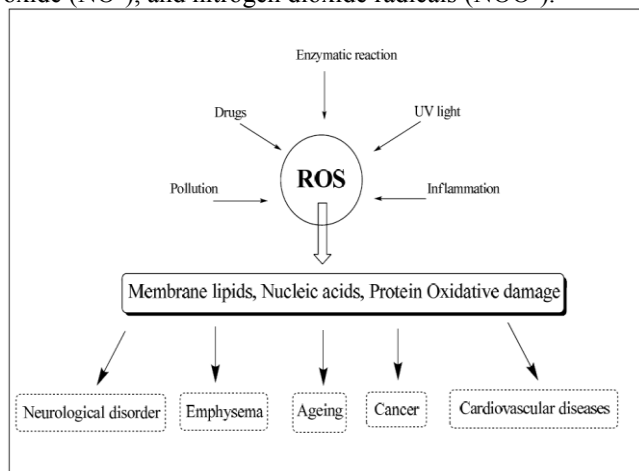
- The role of oxidative stress in human diseases.
- The antioxidant structure-activity relationship of flavonoids.
- The structure characteristics of an effective flavonoid antioxidant.

The role of oxidative stress in human diseases

Oxidation is the transfer of electrons from one atom to another. It represents an essential part of our metabolism and aerobic life in general, since oxygen is the ultimate electron acceptor in the electron flow systems that transport energy in the form of ATP. Problems may arise however when the electron flow generates free radicals, such as O₂-centred free radicals, known as reactive oxygen species (ROS), and including superoxide (O₂^{•-}), peroxy (ROO[•]), alkoxy (RO[•]), hydroxyl (HO[•]) and nitric oxide (NO[•]) radicals (Barry H, 2001).

The contribution of free radical-mediated processes to the pathogenesis of human disease is indicated by biomarkers of oxidative damage to lipids, proteins, and DNA (**Fig 6**). Such markers have been identified in patients with atherosclerosis, certain cancers, neurodegenerative diseases, and lung disorders, especially those with an inflammatory component to their etiology. A range of reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been implicated in the mechanisms of damage associated with disease development, including superoxide radical (O₂^{•-}), hydrogen peroxide (HOO[•]), hypochlorite radical (ClO[•]), ferryl heme protein species, lipid alkoxy (RO[•]) and

peroxyl radicals (ROO^\cdot), peroxyxynitrite (ONOO^-), nitric oxide (NO^\cdot), and nitrogen dioxide radicals (NOO^\cdot).



Impact of ROS on Human body

ROS may be very damaging, since they can attack lipids in cell membranes, proteins in tissues or enzymes, carbohydrates and DNA to induce oxidative modifications, which cause membrane damage, loss of protein function and DNA damage. This oxidative damage is considered to play a causative role in ageing and several degenerative diseases associated with it, such as heart disease, congestive dysfunction and cancer. Humans have evolved antioxidant systems to protect against free radicals. These systems include some antioxidants produced in the body (endogenous antioxidants) and others obtained from the diet (exogenous antioxidants).

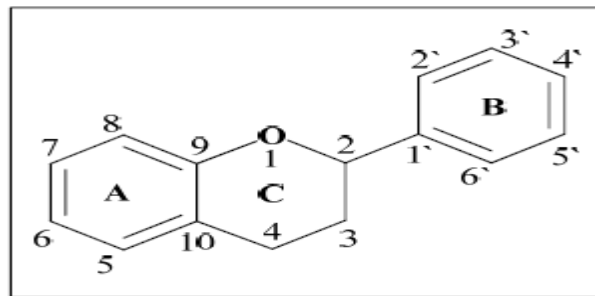
Defense systems against damage induced by ROS fall into three categories:

- Preventative antioxidants that suppress free radical formation.
- Radical-scavenging antioxidants that inhibit initiation of chain reactions and intercept chain propagation, including catalytic antioxidants. Antioxidants involved in repair processes.

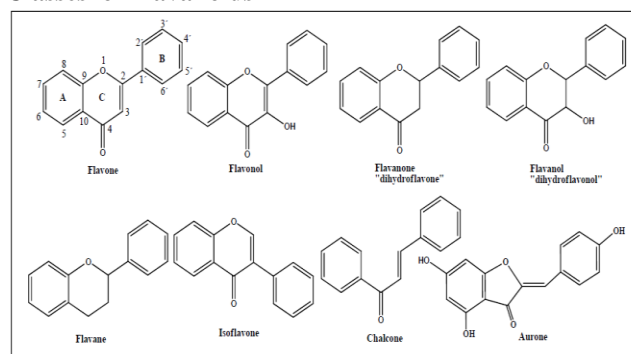
The antioxidant structure-activity relationship of flavonoids

Flavonoids are polyphenolic substances that are based on the flavan nucleus which consists of 15 carbon atoms arranged in three rings (C6–C3–C6) (*fig – given below*). The rings are labelled A, B and C (Bauerova K and Bezek S, 1999).

The major flavonoid classes include flavonols, flavones, flavanones, isoflavones flavanols, chalcones and aurones. Differences between the flavonoid classes are mostly limited to the pyrone ring (absence or presence of double bond, presence of 3-hydroxy and/or 4-oxy groups) and in the number of hydroxyl groups in rings **A** and **B**.



Classes of flavanoids



Flavonoids may be monomeric, dimeric, or oligomeric and vary greatly in molecular weight. Polymeric derivatives, called tannins, are divided into two groups relating to their structure, *i.e.* condensed and hydrolysable. Condensed tannins are polymers of flavonoids and hydrolysable tannins contain gallic acid.

The chemistry of the flavonoids is predictive of their free radical scavenging activity as the reduction potentials of flavonoids and the consequently radical form, are lower than those of alkyl peroxyl radicals and the superoxide radical, which therefore means the flavonoids may inactivate these radical species and prevent the deleterious consequences of their reactions (Hanasaki Y *et al.*, 1994; Tsajimoto Y *et al.*, 1993; Jovanovic S *et al.*, 1996; Dr Navneet S, 2014; Harman D, 1956; Amit K and Priyadarsini KI, 2011).

The electron/H-donating properties of flavonoids are considered to be the basis of their antioxidant action. Their free radical scavenging properties are best approached through structure-antioxidant activity relationships. The ability of flavonoids to act as antioxidants by electron donation depends directly on the reduction potentials, and inversely on the reactivity of the flavonoid molecules with dioxygen, as the generation of peroxyl radicals will propagate oxidative reactions. These concepts have been reviewed (Dr Navneet S, 2014).

Preparation of extract

The extract were received from Botany and Cytogenetics department of Wadia College, Pune. Part used – petals of flowers.

MATERIALS AND METHODS

A modification of thiobarbituric acid reactive substances (TBARS) assay was used to determine the level of lipid peroxide formed using egg yolk homogenate as lipid-rich media. Egg homogenate (0.5 ml, 10% v/v) will be added to 0.1 ml of extract (1mg/ml) and the volume made up to 1 ml with distilled water. Then, 0.05 ml of FeSO₄ will be added and the mixture incubated for 30 minutes. Acetic acid (1.5 ml) and thiobarbituric acid (1.5 ml) in SDS will be sequentially added. The resulting mixture will be vortexed and heated at 95°C for 60 minutes. After cooling, 5 ml of butanol will be added and the mixture centrifuged at 3000 rpm for 10 minutes (Harman D, 1956). The absorbance of the organic upper

layer will be measured at 532 nm and converted to percentage inhibition using the formula:

$$\text{Inhibition of lipid peroxidation (\%)} = (1 - E/C) \times 100$$

Where C = absorbance of fully oxidized control and E = absorbance in the presence of extract

TK100 –Drug prepared from bark of *Delonix regia*

TK101- Drug prepared from Flowers of *Delonix regia*

Analysis by student T TEST

$$t=4.3$$

$$df=6+6-2=10$$

T VALUE at df 10 is 4.3 lies beyond the recorded value 3.169 at probability level 0.01, which indicate that the difference in the mean inhibitory capacity of two drug significant (P<0.01).

Table 1. Isolated flavanoids from *Delonix regia*

Compound	R	R ₁
Cyanidin 3-O-glucoside	H	OH
Cyanidin 3-O-rutinoside	Rhamnoside	H
Pelargonidin 3-O-rutinoside	Rhamnoside	OH
Cyanidin 3-O-gentiobioside	Glucoside	OH

Table 2. Isolated flavanoids from *Delonix regia*

Compound	R	R ₁	R ₂
Kaempferol -3-O- glucoside	H	H	H
Kaempferol -3-O-rutinoside	H	Rhamnoside	H
Kaempferol -3-O-rhamnosyl neohesperidoside	H	Rhamnoside	Rhamnoside
Kaempferol -3-O- neohesperidoside	H	H	Rhamnoside
Quercetin -3-O-rutinoside	OH	Rhamnoside	H

Table 3. Inhibition of Lipid Peroxidation

TK100	TK101
43.97	58.56
45.877	62.15
56.23	69.344
59.83	73.15
61.31	80.126
64.05	83.509

TK100 –Drug prepared from bark of *Delonix regia*; TK101- Drug prepared from Flowers of *Delonix regia*

Figure 1. Bar Graph

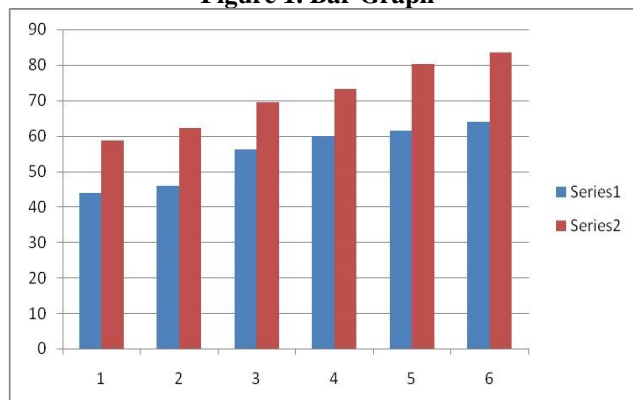
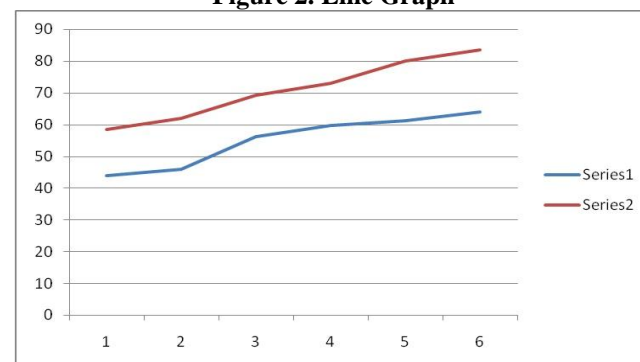


Figure 2. Line Graph



BLUE –TK100, RED-TK101

CONCLUSION

TK101 has significant inhibition on lipid peroxidation when compared with TK100 further studied is required to explore the property in vivo.

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