International Journal of New Technology and Research (IJNTR)
ISSN:2454-4116, Volume-2, Issue-1, January 2016 Pages 80-88

Comprehensive Standardization And Assessment of Behavioural Core In Rodants Of Ethanolic Extract Of Floral Part of Delonix regia (bojer ex hook.) Raf. Of Pakistan

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Abstract— ***Delonix regia* is branched, broad, spreading, flat-crowned deciduous tree and it is well known for its brilliant display of red- orange bloom in the form of bunch. The extract of different parts of plant has been used in the treatment of malaria, bacterial infections and constipation. The present investigation was conducted to standardize the drug for quality assessment and in addition to evaluate its behavioural core in rodent. In standardization of plant material the pharmacognostic techniques (Macroscopic, microscopic, histological characteristics, Physico chemical parameters, ash values along with extractive values) was carried out. Macroscopic studies indicated the presence of five thick crimson sepals curve back to display their lime- green lining and five spoons shaped petals, one of them is larger having streaked centre. Microscopically, the transverse section of Delonix regia flower showed the presence of single layer of barrel shaped cells with stomata. Cells containing colouring pigments were also present. Powdered flowers material revealed the presence of Oil cells, Fibres, Fragments of vascular tissues, ca-oxalate crystals, starch granules, cork tissues epidermal and stone cells. Physico chemical parameters including total ash value which was 6.51% w/w, acid insoluble ash was not more than 8.4% w/w, water soluble ash is equivalent to 7.44% w/w and sulphated ash is 16.76 % w/w. Alcohol soluble extractive value was more than water soluble extractive value. Phytochemical analysis showed presence of steroids, alkaloids, flavonoids, proteins, tannins, carbohydrates, phenol and triterpenes. Infrared spectroscopic analysis revealed the presence of O-H, C-H, N-H, C=O, C-N and C-O. In case of behavioural study, rotarod showed considerable lack in motor coordination at 100 mg / kg on 30 and 60th mins of duration which was 44% and 32.2% respectively while in case of grip strength all tested doses were found ineffective in related to muscle relaxant property**

Index Terms— **Delonix regia, Standardization, Macroscopic, Microscopic, Phytochemical, Physico chemical parameters, Behavioral core..**

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I. INTRODUCTION

Standardization is a fundamental requirement for the whole plant, parts of plant or extracts in order to evaluate the quality of drugs [1]. In recent years rapid increase has been found in the standardization of medicinal plants having potential therapeutic significance [2 & 3]. The world health organization estimates that about 80% of people living in developed countries more or less dependent on traditional medicines for their primary health care needs. People under developed countries utilize medicinal plant on regular basis as medicinal plants are the backbone of the traditional medicines [4]. Therefore, there is widespread interest in drugs derived from plants. The shortcomings of the drugs available today, propel the discovery of new pharmacotherapeutic agents in medicinal plants [5].

same time and places but in different years and with different environmental factors surrounding the cultivation of a particular medicinal plant. That's why it is very important that a system of standardization is established for every plant medicine [6].

Behavioral research has considerable contribution to the understanding, treatment, and prevention of behavior and brain disorders. Psychological and biological information across species were evaluated by animals as experimental models. Because of this permanence, use of animals in research for behavioral procedures has led to several advances in knowledge and has beneficial for both humans and animals [7]. *Delonix regia* (bojer) belongs to family *Leguminosae* and is commonly known as royal poinciana. This plant is native of Madagascar, now cultivated in West Pakistan, Arabia, India, Abyssinia and Nubia [8]. Royal poinciana is used for ornamental purpose [9]. It is a large deciduous tree, 12-17 m high. Leaves are bipinnate, up to 60 cm long, pinnae 11-18 pairs, leaflets 20-30 pairs on each pinna, oblong, 7.5 - 10 mm long and 3.5 - 4 mm wide. Flowers are up to 8 cm long, bright red in colour having 5 Sepals and petals. The fifth upright petal called standard, which is slightly larger having yellow or white streaked with red. The flowering period of plant is from April to August. Pods are 30-50 cm long, 5 cm broad, compressed and firm. Seeds are 20–40 cm long, oblong, transverse, and mottled [10]. The plant is used to treat constipation, inflammation, arthiritis and hemiplagia [11]. Its seeds contain a gum

therefore utilized in food and textile industries [12]. The bark is used as antiperiodic and febrifuge [11]. Flowers are used in dysmenorrhoea [9].

The present study was carried out for the standardization of the floral part of *Delonix regia* of Pakistan by means of pharmacognostical characteristics (Macro and microscopy, physicochemical and phytochemical) because these parameters are helpful for the future identification and authentification of the plant material. Furthermore rota rod and grip strength tests were also carried out in order to assess the behavioural core in rodents.

II. MATERIALS AND METHODS:

A. Plant material:

Delonix regia (Bojer) flowers (2.0 kg) were soaked in a container with 3 liter of ethanol (Merck). After keeping 15days the plant was percolated with Whatmann filter paper No 1. Filtrate was evaporated to semisolid residue (164 g) on a rotary evaporator at 32oC (Fig 1). The plant was collected from University premises Karachi in the month of May and authenticated by Prof. Dr. Ghazala. H. Rizwani, Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi and voucher specimen is deposited in the Herbal museum of the Department of Pharmacognosy with number 00133 for further reference.

B. Instruments used:

Nikon Optiphot with Camera Fx35 WA, Lens 2.5mm, Drying oven (DHG- 9053A), Muffle furnance 1000 0 C (A product of PCSIR, MF- 102), UV cabinet (Toshiba India Model B) and FT-IR Spectrophotometer (Thermo Spectronic Model, Heliose Alpha No UVA 090714, England).

C. Macro and microscopic studies:

The morphological characters of the flower were described based on the shape, size, colour, odour and texture. The Transverse sections of the flower was cut by free hand sectioning and selected the finest and complete section after completion of mounting procedure. Various histological features and their type, shape, size and arrangement were observed in the sliced drug material with the help of microscope. Powdered flower was passing through sieve no 60 for obtaining fine quality of powder and mounted in three reagents 10% chloral hydrate, 5% iodine and 50% aqueous glycerine solutions for identification of the major diagnostic features of cell fragments present in the plant material [13].

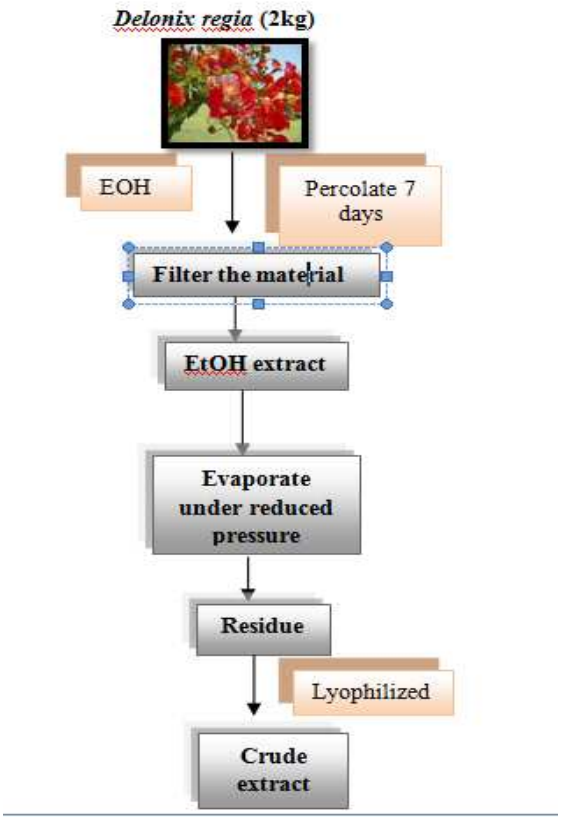


Figure1. Extraction of plant drug

D. Physico chemical analysis:

Physico chemical parameters [14] and Extractive values of sample with different solvents were also determined [15].

E. Preliminary phyto chemical screening:

Small quantity of ethanolic extract was taken and added different reagents to detect the presence or absence of different phytoconstituents such as steroids (Salvoski's test), Alkaloids (Dragendroff test), tannins (Lead acetate test) etc by usual prescribed methods [16].

F. Solubility test:

Powdered drug (1g) was mixed in 4 ml of various solvents separately. Then each test tube was shaken and boiled. The maintenance of original color of powdered materials was noted in different solvents in cold and hot settings. Change in color was found through the filter paper [17]

G. Florescence analysis:

Fluorescence analysis of the powdered flowers was also carried out through standard method [18].

H. Infrared spectroscopy analysis:

The oven dried plant parts were ground into a fine powder and the FT-IR spectra were recorded by using FT-IR Spectrophotometer.

I. Rota rod test:

It was conducted for the consideration of neurological deficit in mice treated with ethanolic extract of floral part of plant. Each mice was placed on a rod (32 mm diameter) for three consecutive trials and the rod is driven by a motor with 5 rpm rotational speed. The group of each animal was then placed on the rod at an interval of 0, 30, 60, 90 and 120 mins and the mice able to remain on the top for 3 mins were selected for the study [19].

J. Grip strength:

Muscles strength property of mice was estimated by grip strength test [20]. The animal's forelimbs grip strength a measure in the apparatus by a bar connected to a force transducer and is measured in grams. Infront of grasping trapezene each group of animal was then placed over a plate which is joined to peak amplifier. When animal was pulled by the tail, the animal grasps the trapezene. After the animal loses its grip on the grasping trapezene, the peak preamplifier automatically store the peaks pull force.

K. Statistical analysis:

The results were expressed as Mean ±SEM. The data were analyzed by ANOVA (version 15.00) followed by Tukey post hoc test. $p<0.05$ was considered as statistically significant.

Colour	Orangish red
Taste	Unpleasant
Odour	Characteristic
Shape and Size	Each flower is about 12.5 cm across, five thick crimson sepals curve back to display their lime-green lining. From the spaces between them radiate the five spoons shaped, wavy and crinckle edged petals, one of them is larger, its white centre is streaked and splashed with scarlet
Texture	Smooth

Table 2. Powdered analysis of flower of *Delonix regia*

Powder analysis	
Powder characteristics	**Observation**
Colour	Brownish red
Taste	Unpleasent
Odour	Characteristic
Texture	Rough

III. RESULTS

Organoleptical characters (Table 1 and 2) play an important role in the identification of crude drugs. The transverse section (Fig 2) of flower of Delonix regia shows that Upper epidermis having single layer of barrel shaped cells covered with a thick cuticle. Stomata are seen at regular intervals. Below upper epidermis single layer of cells containing colouring pigments were also present. Middle portion is composed of many layers of cortical cells. Above the lower epidermis double layers of cells containing colouring pigments were also present. Powdered flower of Delonix regia showed the occurrence of

Table 1. Macroscopic study of flower of *Delonix regia*

Macroscopic study	
Organoleptic Characters	**Observation**

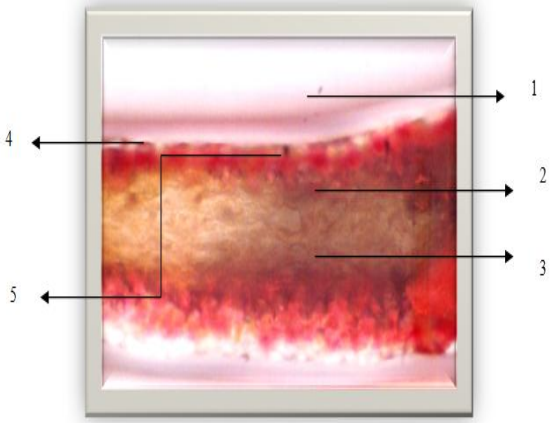


Figure 2. T. S of flower of *Delonix regia* (Bojer ex Hook)Raf.

1. Upper epidermis, 2. Cortical cells, 3.Cells having coloring pigments, 4. Barrel shaped cells, 5. Stomatal chamber

Spherical and double layers Oil cells. Fibres are simple and some portion having lignin deposition. Thick and elongated phloem fibres were also present. Fragments of vascular tissues and Cells containing tannins and colouring pigments were present. Epidermal cells were also present. Stone cells having narrow lumen with thick lignin deposition were present. Rosette shape Ca-oxalate crystals and Abundant Simple, oval or spherical and compound types starch granules were seen in powder sample. Cork tissues were present in powder material (Fig 3 and Table 3)

The preliminary phytochemical screening was performed which showed the presence of various active phytoconstituents (Table 4). The powdered leaf, seed and flower of all three drugs (I, II and III) were slightly soluble only in sulphuric acid. They did not retain their original colors in various solvents by cold and hot tests (Tables 5). The powdered drugs of I, II and III did not retain its original color in different solvent on dry filter paper (Tables 6). Physicochemical parameters such as percentage of loss of weight on drying, Moisture content, dry matter weight, total ash, water-soluble ash, acid insoluble-ash and sulphated ash were determined. The percentage of extractive values in ethanol and water were also determined (Fig 4). The powdered material of floral part of Delonix regia in various solvents was examined under both ordinary light and UV light (Table 7)

The IR spectra of Delonix regia showed an intense broad band of the O-H group at 3244 cm-1. The C-H (aromatic and aliphatic) group peaks appear at 2925 and 869 cm-1respectively. The peak was observed at 2357 cm-1 due to N-H stretching. The Stretching vibration of the C=O was appeared at 1687 cm-1

Fig 3 (a): Stone Cells

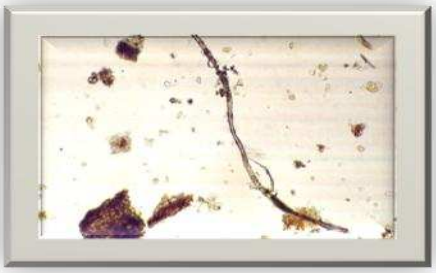


Fig 3(b): Phloem Fiber, Cork Tissues, Starch Granules and Ca-oxalate Crystals

Fig 3(c): Vascular tissues, Fiber, Oil Cell and Epidermal Cells

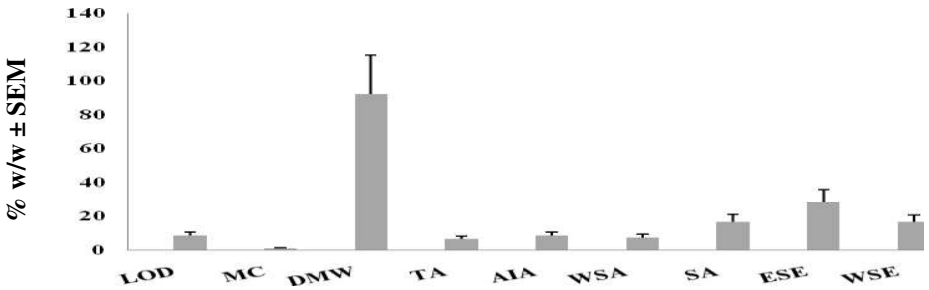
Fig 3(d): Sheet of Stone Cells

Figure 3. Powder analysis of *Delonix regia* (Bojer ex Hook)Raf

Table 3. Microscopic evaluation of powdered flower of *Delonix regia*

+ Present, - Absent

S.No	Reagents	Colour/precipitate	Constituent	Inference
1.	Salvoski' s test	Purple ring appears	Steroids	+
2	Dragendroff reagent	Orange precipitates	Alkaloids	+
3	Ammonia III sulphate solution 0.2% in water	Brown colour	Flavonoids	++
4	Picric acid solution	precepitation	protein	+
5	Lead acetate test	precepitation	tannins	+
6	Frothing test	No foam observed	Saponins	-
7	Molisch reagent	Purple color at the junction	carbohydrates	+
8	Ferric chloride test	Blue color	phenol	+
9	Lieberman's burchard test	Pink color	triterpenes	+



LOD: Loss on drying, MC:Moisture Content, DMW: Dry matter weight, TA: Total ash,
AIA: Acid insoluble ash, WSA: Water soluble ash, SA:Sulphated ash,
ESE: Ethanol soluble extractive, WSE: Water soluble extractive

Figure 4. Quantitative standards for powdered flower of *Delonix regia*

Table 4. Phytochemical screening of ethanolic extract of flower of *Delonix regia*

S.No	Solvents	Volume	*Delonix regia* Bojer Ex Hook Raf (flowers)			
			Cold Test		**Hot Test**	
			Colour	**Solubility**	**Colour**	**Solubility**
1	**Acetone**	4ml	Reddish brown	Insoluble	Reddish brown	Insoluble
2	**Acetic acid**	4ml	Brownish yellow	Insoluble	Brownish red	Insoluble
3	**Butanol**	4ml	Reddish brown	Insoluble	Reddish brown	Insoluble
4	**Chloroform**	4ml	Reddish brown	Insoluble	Brownish red	Insoluble
5	**Ethanol**	4ml	Reddish yellow	Insoluble	Reddish brown	Insoluble
6	**Hexane**	4ml	Reddish yellow	Insoluble	Reddish yellow	Insoluble
7	**Methanol**	4ml	Reddish brown	Insoluble	Brownish orange	Insoluble
8	**Sulphuric acid**	4ml	Dark brown	Insoluble	Dark brown	Slightly soluble
9	**Water**	4ml	Reddish brown	Insoluble	Reddish brown	Insoluble

++ Moderate, +Light,

Table 5. Solubility and Color Analysis of Powdered Flower of III in Various Solvents by Cold and Hot methods

S.No	Solvents	Volume		*Delonix regia* Bojer Ex Hook Raf (flowers)			
				Cold Test		**Hot Test**	
				Colour	**Solubility**	**Colour**	**Solubility**
1	**Acetone**	4ml		Reddish brown	Insoluble	Reddish brown	Insoluble
2	**Acetic acid**	4ml		Brownish yellow	Insoluble	Brownish red	Insoluble
3	**Butanol**	4ml		Reddish brown	Insoluble	Reddish brown	Insoluble
4	**Chloroform**	4ml		Reddish brown	Insoluble	Brownish red	Insoluble
5	**Ethanol**	4ml		Reddish yellow	Insoluble	Reddish brown	Insoluble
6	**Hexane**	4ml		Reddish yellow	Insoluble	Reddish yellow	Insoluble
7	**Methanol**	4ml		Reddish brown	Insoluble	Brownish orange	Insoluble
8	**Sulphuric acid**	4ml		Dark brown	Insoluble	Dark brown	Slightly soluble
9	**Water**	4ml		Reddish brown	Insoluble	Reddish brown	Insoluble

Table 6. Colour analysis of powdered drug of *Delonix regia* bojer ex hook raf in various solvents with known volume by filter paper

S.No	Solvents	*Delonix regia*			
		Volume	**Actual colour of powdered drug**	**Colour in solvents**	**Colour on filter paper**
1	**Acetone**	4ml	Reddish brown	Reddish brown	Light yellow
2	**Acetic acid**	4ml	Reddish brown	Brownish yellow	No colour
3	**Butanol**	4ml	Reddish brown	Reddish brown	No colour
4	**Chloroform**	4ml	Reddish brown	Reddish brown	No colour
5	**Ethanol**	4ml	Reddish brown	Reddish yellow	No colour
6	**Ethyl acetate**	4ml	Reddish brown	Reddish brown	No colour
7	**Hexane**	4ml	Reddish brown	Reddish yellow	No colour
8	**Methanol**	4ml	Reddish brown	Reddish brown	No colour
9	**Sulphuric acid**	4ml	Reddish brown	Dark brown	Brown
10	**Water**	4ml	Reddish brown	Reddish brown	No colour

Table 7. Florescence analysis of powdered flower of *Delonix regia*

Sample	Color in day light	Color in short uv	Color in long uv
powder	Brownish red	Dark green	Brownish red
Powder+1N NaOH in methanol	Brownish red	Dull green	Reddish black
Powder+1N NaOH in water	Brownish red	Green	Brownish black
Powder+1N Hcl	Brownish red	Green	Brownish black
Powder+50% HNO_3	Brownish red	Green	Brownish red
Powder+50%H_2SO_4	Brownish red	Green	Brownish red

The band observed at 1102 cm^{-1} represents C-N stretching and the C-O stretching was observed at 1002 cm^{-1} (Table 8 and Fig 5)**.**

Table 8. The IR spectroscopic analysis gave the following characteristics absorption peaks of ethanolic extract of flowers of *Delonix regia*

S.No	Components	Wavenumbers (cm^{-1})
1	**O-H**	**3300**
2	**C-H**	**2915.30, 2872.35**
3	**N-H**	**2357.32**
4	**C=O**	**1748.61**
5	**C-N**	**1236.85**
6	**C-O**	**1043.59**

Floral extract showed significant lack ($p < 0.01$) in motor coordination at 100 mg / kg on 30 and 60th mins of duration which was 44% and 32.2% respectively whilst the diazepam (the standard reference drug) significantly reduced ($p < 0.01$ and $p < 0.001$) time span (29% and 9.3%) as compared to control group at the 30 and 90th mins respectively (Table 9).

Extract showed significant ($p < 0.05$) increased in muscle strength after 90 mins and lasted for 120 mins at dose of 100 mg / kg as compared to control which were 75% and 120% respectively. The reference drug Diazepam (5mg / kg) represented reduction (24% and 14%) in muscle strength at a time interval of 60 and 120 mins as compared to control, thus indicating myorelaxant effect (Fig 6).

IV. DISCUSSION

Traditional medicine has remained as the most affordable and easily available source of treatment in the primary healthcare system. But there is a lack of accurate method for their standardization and evaluation Therefore there is a need of documentation of research work carried out on traditional medicines. The process of standardization is achieved by stepwise pharmacognostic studies [6]. These studies help in correct identification and authentication of plant material. According to WHO the microscopic description is an important step towards establishing the identity and purity of medicinal plant and should be carried out before any tests are undertaken [21].

Crude drug contains different primary and secondary metabolites. Generally occurrences of secondary metabolites in plant material possess medicinal properties. Therefore detailed screening is required to isolate active constituents so, that it may be scientifically proved to access the pharmacological responses of the plant to ascertain its uses. The wide range of phytochemical compounds was detected in *Delonix regia* having great medicinal significance.

Solubility is also an important parameter for preliminary evaluation of particular drug. Different terms indicate solubility for example less than 1 part (very soluble), 1–10 parts (freely soluble), 30–100 parts (sparingly soluble) etc [22]. The powdered material was slightly soluble only in sulphuric acid and it did not maintain its original color in different solvents by cold and hot treatments. The use of FTIR fingerprinting for plant extract tends to focus on identification of different functional groups and their arrangement in the molecule of specific compound which is useful for evaluation of drug.

Behavioural core provides planning and analysis of behavioral activity coordinated movement, learning and memory, fretfulness, depression, convulsion susceptibility and aggression in rodents. Rotarod test was conducted for assessment of motor coordination and in order to ascertain its effect on possible neuromuscular inhibition [23 & 24]. *Delonix regia* showed significant lack in motor coordination at 100 mg / kg on 30 and 60th mins of duration which was 44% and 32.2% respectively.

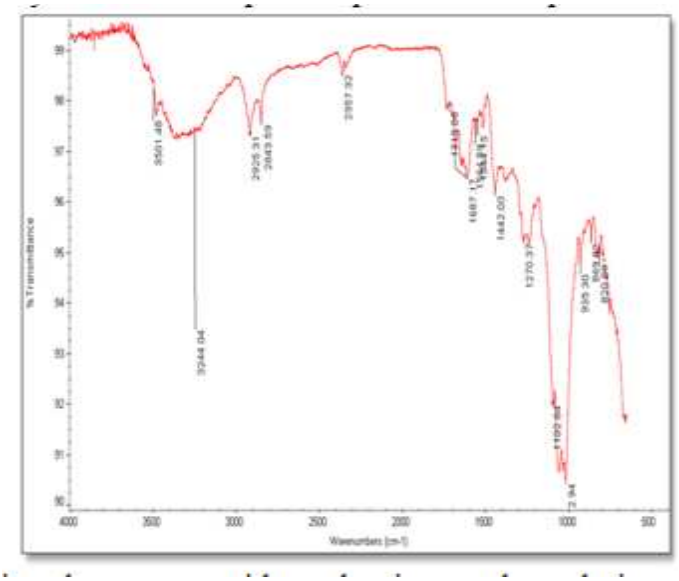


Figure 5.The IR spectroscopic analysis of EtOH extract of flowers of *Delonix regia* (Bojer ex Hook) Raf

Table 9. Effect of the EtOH extract of the flowers of *Delonix regia* on motor coordination in mice

Treatments	Time spend on rotarod at				
	0 min	**30mins**	**60mins**	**90mins**	**120mins**
DR(100mg/kg)	180±0	101.66±13.64**	122 ±11.80**	180±0	180±0
DR(200mg/kg)	180±0	172.33±7.66	180±0	180±0	180±0
Diazepam (5mg/kg)	180±0	126.33± 17.32**	163.33 ± 8.81	143.33 ± 6.00***	180±0
Control (10ml/kg)	180±0	180±0	180±0	180±0	180±0

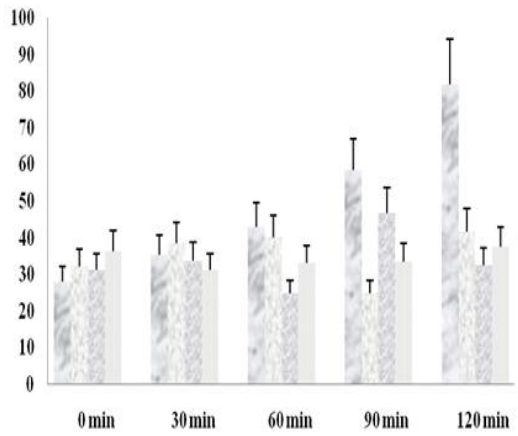


Figure 6 Time Profile of the myorelxant effect of delonix regia

Grip Strength is being used to evaluate muscular strength or neuromuscular function in rodents [20]. The tested plant extract showed significant increased in muscle strength after 90 mins and lasted for 120 mins at dose of 100 mg / kg as compared to control which were 75% and 120% respectively.

V. CONCLUSION:

Macro and microscopic studies, preliminary phytochemical screening, physicochemical parameters, solubility test, fluorescence and Infrared spectroscopic analysis used as a diagnostic features for the correct identification of Delonix *regia.* Hence, these standardization parameters are useful in detecting the adulterants and will lead to efficacy and purity of the selected plant. Behavioural experiments conclude that ethanolic extract of *Delonix regia* showed some neuromuscular inhibition and having significant muscles stimulant property.

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