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Original Research Article

PHYTOCHEMICAL SCREENING AND *IN-VITRO* ANTIOXIDANT ACTIVITY OF METHANOL EXTRACT OF *Delonix regia* (HOOK) SEED PODIUM.

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ABSTRACT

Delonix regia (hook) commonly known as "flame of forest" is a plant globally distributed around Madagascar, India, Africa and Australia. Various parts of this plant are traditionally used for the treatment of inflammation, rheumatism, bronchitis, diabetes, anaemia, fever and pneumonia. The seed podium used in this study is extracted with petroleum benzene, chloroform, and methanol. The methanolic extract shows the presence of highest amount of phytoconstituents and it is further processed for *in vitro* anti-oxidant activity. The methanol extract shows a potential in vitro anti oxidant capacity against DPPH (2, 2- di-phenyl-1-picrylhydrazine), Nitric oxide, Hydrogen peroxide and also shows reducing power when compared with ascorbic acid as standard.

Key words: *Delonix regia*, anti-oxidant, DPPH (2, 2- di-phenyl-1-picrylhydrazine), phytoconstituents, *in-vitro*.

INTRODUCTION:

Medicinal herbs are growing abundantly from fringe to mainstream with a greater source of health remedies which are free from side effects caused by synthetic chemicals (Abdille *et al*., 2005). Now a day's more emphasis has been given to use eco-friendly and bio-friendly plant based medicines rather than synthetic drugs for human diseases. In the last hundred years plants have become an important source for the discovery of novel pharmaceuticals with many blockbuster drugs being directly or indirectly derived from plants (Choudhury *et al.,* 2017). Many synthetic anti-oxidants are also easily available in market but these agents are having more or less side effects whereas plant derived anti-oxidants shows greater potency with almost negligible side effects (Sayed *et al.*, 2011). Anti-oxidants are essential for the regulation of cellular redox state which is responsible for the well-being of a single cell or whole organism (Kumar *et al.,* 2019). When the cellular redox state cannot deal with the reactive oxygen species that is (ROS) and the cells are being placed on the oxidative stress and cells will suffer from scarcity of oxygen and it can effectively damage the cellular protein, lipids and ultimately leads to cell death. Excessive oxidative stress leads to numerous health issues and numerous disease like neurodegenerative disease, cancer, diabetes atherosclerosis, cirrhosis and auto-immune disease (Kokate *et al.,* 2012). Cells maintain the redox state by balancing the level of oxidants and antioxidant by both enzymatic and non-enzymatic mechanisms (Krishna *et al.,* 2012; Kunle *et al.,* 2012). High anti-oxidant yielding herbal medicines contains high level of phenolic and Flavonoid compounds which can scavenge the free radical which are known as reactive oxygen species (ROS). *Delonix regia* (Hook) is a flowering plant with long branches spreading in umbrella shape. It is commonly known as flamboyant, radhachura, krishnachura, gulmohar. It belongs to the bean family Fabaceae. It is known by its fern like leaves and flamboyant display of orange-red flowers over summer. In most of the tropical regions of the world it is known as ornamental tree and in English it is given the name as "flame of the forest"(Malsawmtluangi *et al.,* 2014).

MATERIALS AND METHODS

Collection and Processing Of Plant Materials

The fresh seed podium of *Delonix regia* was collected from the north-eastern state Mizoram, India during the month of July and August. The plant materials were initially identified by its morphological character and

the plant was authenticated by Dr. Chaya Deori scientist-D, In-charge Botanical Survey of India, eastern circle, Shillong with reference no-BSI/ERC/Tech/2019-20/720. The collected plant materials are then washed and cut into small pieces and dried under shed (Modi *et al.*, 2016).

Preparation of Extract:

The dried plant materials are ground into powder, then the powdered seed podium (1kg) packed within a thimble and subjected to successive extraction by different solvents with increasing polarity like petroleum benzene, chloroform and methanol using a Soxhlet apparatus (Noumiu and Dibakto, 2000; Omer *et al.*, 2019). The extraction was run for around 72 hours for each solvent or till the solvent in siphon tube becomes colorless (Prakash *et al*., 2013). The solvents were recovered by distillation under reduced pressure using rotary vacuum evaporator to obtain crude extract. The plant extract was preserved in refrigerator and the methanol extract was used to determine the biological activities (Ravinder *et al.*, 2018).

Preliminary Phytochemical Screening:

The crude methanolic extract of *Delonix regia* was subjected to preliminary phytochemical screening with the help of pre-established procedures (Seal and Chaudhry, 2015). The extract was analyzed for the presence of saponin, phenolic compounds, Flavonoids, phytosterols, triterpenoids, reducing sugars, cardiac glycosides, anthraquinones, tannins and alkaloids by following well established procedure (Shad *et al.*, 2013).

EVALUATION OF IN-VITRO ANTI-OXIDANT CAPACITY:

Determination of Total Phenolic Compound: -

The total phenolic compound present in methanolic extract of *Delonix regia* seed podium were determined by using spectrophotometric method. The total phenol content was determined using Folin-Ciocalteu phenol reagent method. 1 ml of extracts and 9 ml of distilled water were transferred to a 25 ml volumetric flask. To it, 1 ml of Folin-Ciocalteu phenol reagent (previously diluted ten times with distilled water) was added and shaken well. After five minutes 10 ml of 7% Na_2CO_3 solution was added to the mixture. In the same manner, different concentrations of Standard Gallic acid solutions (20, 40, 60, 80 and 100µg/ml) were prepared (Thakur *et al.*, 2018). Both the sample (extract) and standard solutions were incubated for 90 minutes at room temperature. The absorbance of the solutions was taken against the blank at 550 nm using UV-Visible spectrophotometer (Thermo

Fisher Scientific 201). The total phenol content was expressed in GAE (mg/g) of extract (Tai et al., 2012).

Determination of Total Flavonoid Content:

1 ml of methanolic extract of *Delonix regia* seed podium is mixed with 3 ml of 5% sodium nitrate along with 0.3 ml aluminum chloride. After 6 minutes added 2 ml of NaOH. Adjust the volume up to 10 ml with the solvent used for extract preparation. Then absorbance was measured at 510 nm by using uv-visible spectrophotometer (thermo fisher scientific 201). Quercetin was used as a standard (Singh *et al.,* 2017).

Determination of 2, 2-Diphenyl-1-Picrylhydrazylradical (DPPH) Scavenging Activity:

The free radical scavenging activity of the plant samples and ascorbic acid as positive control was determined using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl). Different concentration of the tested samples were placed in test tubes and mixed with 3.9 ml of freshly prepared DPPH solution (25 mg L^{-1}) in methanol. The absorbance was measured at 517 nm using UV-visible spectrophotometer (Thermo Fisher Scientific 201) after 30 min. The scavenging activity is calculated by using the following equation: -

DPPH scavenged (%) = {(Ac – At)/Ac} x 100

Where Ac is the absorbance of the control reaction and at is the absorbance in presence of the sample of the extracts (Afolayan and Jimoh, 2009). Then the IC50 value was calculated. The IC_{50} value was defined as the concentration in mg of dry material per ml (mg / ml) that inhibits the formation of DPPH radicals by 50%. Each value was determined from regression equation (Aziz, 2015).

Determination of reducing power:

The reducing power of the methanol extract of plant material of *Delonix regia* seed podium was determined by using ascorbic acid as standard. 1 ml of the extract and 1 ml of the standard with various concentrations (20, 40, 60, 80, and 100µg/ml) were mixed with 2.5 ml of phosphate buffer (6.6 pH) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50°C for 30 minutes. The reaction was stopped by adding 2.5 ml of 10% trichloroacetic acid and the mixture was centrifuged at 3000 rpm for 10 minutes. 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water, and 0.5 ml of 0.1% ferric chloride solution and the absorbance was taken at 700 nm using UV-Visible spectrophotometer (Thermo fisher scientific 201). The higher absorbance of the reaction mixture indicated that the reducing power is increased (Bauer et al., 1966).

Determination of Hydrogen Peroxide Radical Scavenging Activity:

A solution of hydrogen peroxide (40 m mol/L) was prepared in phosphate buffer (pH 7.4). The extract in distilled water (3.4 ml) was added to a hydrogen peroxide solution (0.6 ml, 40 m mol/L). Absorbance was measured at 230 NM 10 min later against a blank solution containing the phosphate buffer without hydrogen peroxide using UV-visible spectrophotometer (Thermo Fisher Scientific 201). The percentage of hydrogen peroxide scavenging by the extract and standard (ascorbic acid) was calculated using the following equation:

Scavenged hydrogen peroxide (%) =

$$Ac - As/Ac \times 100$$

Where, Ac is the absorbance of the control and As is absorbance in the presence of the extract (Breslin and Andrew, 2017).

Nitric Oxide Scavenging Activity: -

0.5ml of sodium nitroprusside solution was mixed with 1 ml of plant extract. The mixture was incubated for 3 hours at 25°c and then freshly prepared Griess reagent was added in equal volume. Absorbance was measured at 546 nm. Ascorbic was taken as reference standard. The percentage of hydrogen peroxide scavenging by the extract and standard (ascorbic acid) was calculated using the following equation:

Scavenged nitric oxide (%) = Ac− As/Ac× 100

Where, Ac is the absorbance of the control and as is absorbance in the presence of the extract (Choudhury et al., 2017).

RESULTS:

Extractive yield:

1kg of powdered *Delonix regia* seed podium were sequentially extracted using Petroleum ether, Chloroform and Methanol and extractive yields were given in Table 1. The concentrated extracts were kept in refrigerator for further use (Deshmukh and Waghmode, 2011).

Table 1: Extractive yield for different solvents.

S.No.	Extract	% yield
1.	Petroleum ether	5.4
2.	Chloroform	7.34
3.	Methanol	15.14

Preliminary Phytochemical Screening:-

All the extract contains a wide range of phytochemical classes. Steroids and Flavonoids are present in all the extracts. Whereas, tannin is present only in methanolic extract. Alkaloids is absent in all the extract, saponin is present in both chloroform and methanolic extract and absent in petroleum benzene extract (Finkel and Holbrook, 2000).

Table 2: Results of preliminary phytochemical screening of all the extract of *Delonix regia* seed podium.

S.No	Chemical constituents	Petroleum benzene extract	Chloroform extract	Methanol extract
1.	Phenolics	-	-	+
2.	Alkaloids	-	-	-
3.	Carbohydrates	+	+	+
4.	Proteins	+	+	+
5.	Steroids	+	+	+
6.	Triterpenoids	+	+	+
7.	Tannins	-	-	+
8.	Flavonoids	+	+	+
9.	Saponins	-	+	+
10.	Glycosides	-	-	-

"+" indicates the presence of the chemical constituents and the "-" indicates that the chemical constituent is absent.

Evaluation of *in vitro* antioxidant activity:

Determination of Total phenolic content:

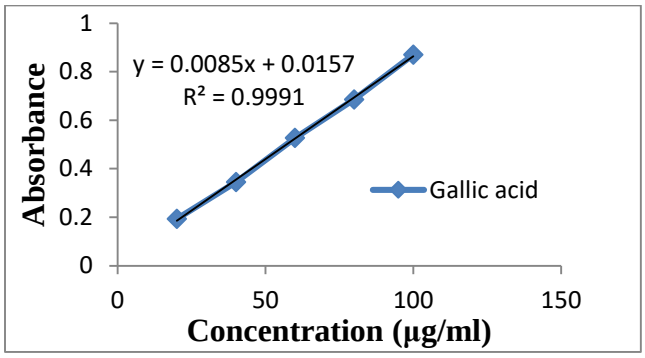

Figure 1:- Standard curve for Gallic acid

Determination of Total Flavonoid Content:

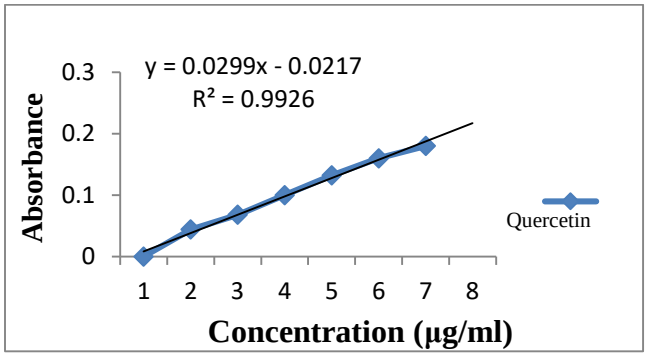

Figure 2:- Standard curve for Quercetin

Determination of DPPH radical scavenging activity:

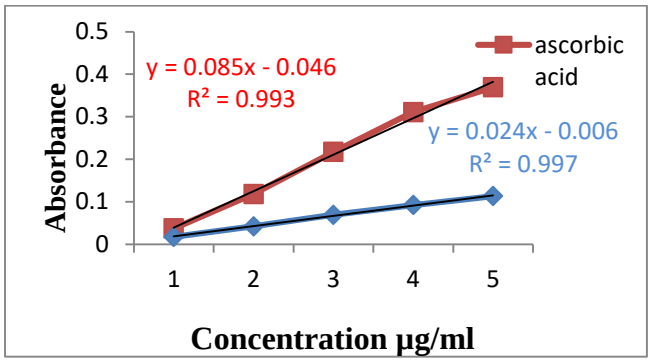

Figure 3:- Standard curve for methanolic extract of *Delonix regia* seed podium and standard ascorbic acid

Determination of reducing power:

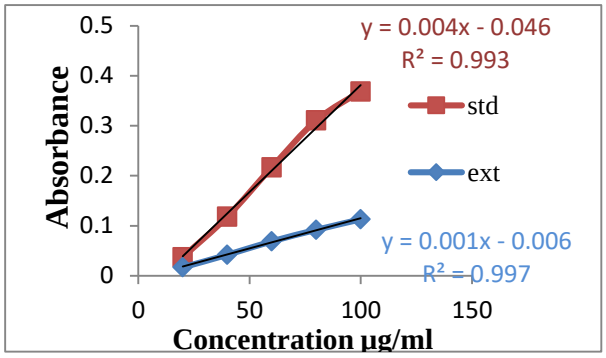

Figure 4:- Reducing power of Methanolic extract of *Delonix regia* and standard ascorbic acid

Determination of hydrogen peroxide radical scavenging activity

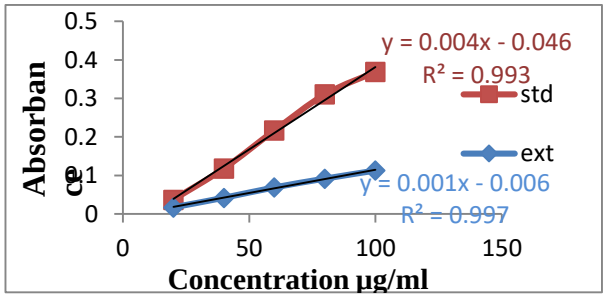

Figure 5: - Standard curve for methanolic extract of *Delonix regia* and ascorbic acid against hydrogen peroxide.

Nitric Oxide Scavenging Activity:-

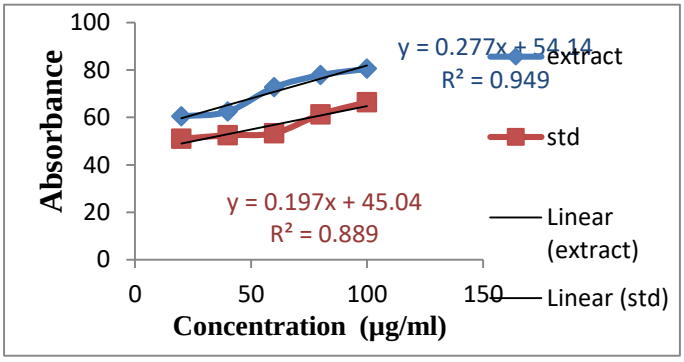

Fig 6:- Standard curve for Standard curve for methanolic extract of *Delonix regia* seed podium and ascorbic acid against nitric oxide.

Table-3:- TPC, TFC value and the IC_{50} values for the methanol extract of *Delonix regia* seed podium.

Assay	Standard	Result
Total phenolic content	Gallic acid	316 ± 0.023 mg/ GAE
Total Flavonoid content	Quercetin	215± 0.034 mg/QE
DPPH scavenging activity	Ascorbic acid	62.42µg/ml
Reducing power	Ascorbic acid	Increases with increase in concentration
Hydrogen peroxide scavenging activity	Ascorbic acid	17.38µg/ml
Nitric oxide scavenging activity	Ascorbic acid	14.84µg/ml

Discussions

Cellular response to oxidative stress occurs in both enzymatic and non-enzymatic mechanisms which will minimize the effect of ROS (reactive oxidative species) (Ghasemzadeh *et al.*, 2012). Increase in intake of non-enzymatic antioxidants can scavenge ROS and can also decrease the probability of the cancer (Gujjeti and Mamidala, 2013). The phytochemicals present in plants either acts as anti-oxidant or pro-oxidant depending upon their concentration, if present in higher concentration acts as an anti-oxidant and if in lower concentration it acts as a pro-oxidant (Hajlaoui *et al.*, 2009). It is also said that plant which are having high amount of anti-oxidant can be used for the prophylaxis of many diseases. Here in this study the plant materials were carried out by successive extraction with different solvents like petroleum ether, chloroform and methanol (Hazarika *et al.*, 2019). The extracts were further used to determine the presence of phytochemical where in it was found that the methanol extract contains the highest amount of phyto constituent of clinical importance (Iqbal *et al.*, 2015). Further the physicochemical parameters of methanol extract were studied to determine its purity and quality (Agarwal and Saini, 2013). The total Phenolic content and Flavonoid content was also estimated which are known for imparting anti-oxidant activity (Kabera *et al.*, 2014). After that the in-vitro anti-oxidant activity was carried out to determine the IC50 value i.e. the inhibitory concentration against DPPH (2, 2-di-phenyl picryl hydrazine), hydrogen peroxide, nitric oxide and the reducing power was also determined taking ascorbic acid as a standard for all the assays. Further studies need to carry out to establish the presented data (Kagithoju et al., 2013).

CONCLUSION

The result of this study shows that the methanolic extract of *Delonix regia* seed podium shows the presence of numerous compounds like Flavonoids, phenolic compound, reducing sugar, carbohydrate and saponin which are responsible for different therapeutic activities (Kar *et al.*, 2012). Flavonoids content present in the extract may protect the cells from oxidative stress by shielding lipid, proteins and DNA from oxidative damage, thereby blocking apoptosis (Kokate *et al.*, 2012). The methanolic extract has been chosen for further study because it contains highest amount of phyto-constituent (Kumpulainen and Salonen, 1999). The methanol extract shows potential anti-oxidant capacity against DPPH free radical scavenging activity, reducing power, hydrogen peroxide scavenging activity and nitric oxide scavenging activity when it's being compared with standard ascorbic acid (Lela, 1981). Furthermore, Bio-activity driven separation studies are required to isolate the active components and determine their mechanism of action (Mishra et al., 2015).

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