CHAPTER – 4

DELONIX REGIA

4.1 Introduction

Delonix regia was discovered in the early 19th century in its native Madagascar by botanist Wensel Bojer (Warren, 2013). It is a species of flowering plant. Initially species was placed in the genus Poinciana, but later on the plant classified in the family of legumes, due to its nitrogen-fixating and soil-improving properties. It is also known as Royal Poinciana or Flamboyant as the leaves of the plant are like ferns and the arrangement of petals are like flamboyant (Parul, 2014). In urdu, hindi and marathi language it is known as gulmohor (Khare, 2007). In Persian gul means petals and mohr means stamp or coin.

Delonix regia is evergreen but in some dry areas it sheds its leaves during the drought. The tree is known for its flowers and in spring as well as in summer season the tree is covered with lively clusters of flame red petals, which covers the tree from May to June (Aridus, 2004). The height of the plant reaches up to 5 to 8 m (Warren, 2013), but its elegant wide spreading umbrella like canopy which can be wider than its height. The appearance of the leaves is feathery and bi-pinnately compound with a characteristic colour which is light but bright green and arrangement is alternate. The leaves of the plant are delicate and are fern like, composed of small individual leaflets, which fold up at the onset of dusk. They are doubly pinnate, oblong and have entire margin. Each leaf is 30 to 50 cm long and has 20 to 40 pairs of primary leaflets or pinnae on it, and each of these is further divided into 10 to 20 pairs of secondary leaflets or pinnules. The *D. regia* provides fullest flowering and best growth when planted in full sun location (Edward *et. al.,* 1993). The petals of the flower grow in corymbs along and at the ends of branches. The colour of the flower is orange or red and comprises of five petals out of which four are spoons shaped, these spreading scarlet grows up to 8 cm in length, whereas the fifth petal is upright and is

Figure No. 4.1: *Delonix regia*

Kingdom	:	Plantae
Division	:	Tracheophyta
Class	:	Magnoliopsida
Order	:	Fabales
Family	:	Fabaceae
Genus	:	*Delonix*
Species	:	*regia*

called the standard, this fifth standard petal is quit larger than the other four and is marked with yellow and white colour. The colour of the seed pods is initially green and is flaccid but turns dark-brown and woody. The length of the seed measures up to 60 cm long and 5 cm wide and the weight is very less weighing an average of 0.4 g. The plant parts taken in this study are its leaves and petals (Aridus, 2004).

4.2 Environmental Requirements and Geographical Distribution

The plant grows in tropical or near-tropical climate, but it also grows in drought and salty conditions. The tree grows in the places rich in organic matter like the loamy soil or free-draining sandy, it does not grow in the places having heavy or clay soils and the growth of petals is more profuse when they get slightly dry climate. The plant of *D. regia* is found almost everywhere in Africa, Caribbean, Cyprus, Hong Kong, the Canary Islands, Mexico, Malta, Northern Australia (South East Queensland, although the plant is now being successfully cultivated in Sydney also in the suburbs of Petersham, Parramatta, Guildford, Warwick Farm and Kurmond). This plant beautifies many other countries like Philippines, Thailand, Taiwan and southern China. The *D. regia* plant is also known as the tree of the city of Tainan, Taiwan, Xiamen, Fujian Province, People's Republic of China and Shantou, Canton Province, People's Republic of China. National Cheng Kung University, a University located in Tainan, and they have put Royal Poinciana on its emblem (Panga, 2014).

Delonix regia is widespread in the western dry deciduous forests of Madagascar, in the wild form it is endangered, but has been widely cultivated in tropical and sub-tropical regions worldwide. The plant now grows in almost all the tropical cities as the seeds of the plant have been carries out all over the world (Aridus, 2004). In the United States, it grows in the South and southwest Florida, the Rio Grande Valley of South Texas, ranging from the low deserts of Southern Arizona (to as high as Tucson), and Southern California. It also grows in the Dominican Republic, Cuba, Haiti, Hawaii, Mexico (especially in the Yucatan peninsula), Nicaragua, Puerto Rico, U.S. Virgin Islands, Guam and the Commonwealth of the Northern Mariana Islands, where it is the official tree of the islands. It is much loved in the Caribbean. It can also be found in the Bahamas. The Poinciana is the national petals of

St. Kitts and Nevis. The island of Mauritius has widespread distribution of the Royal Poinciana where it announces the coming of the New Year. It is a popular street tree in the suburbs of Brisbane, Australia.

4.3 Propagation

The *Delonix regia* is most commonly propagated by seeds. Seeds are collected, soaked in warm water for at least 24 hours, and planted in warm, moist soil in a semi-shaded, sheltered position. Instead of soaking, the seeds can also be knicked or pinched (with a small scissors or nail clipper) and planted immediately. These two methods allow moisture to penetrate the tough outer casing, stibmulating germination. The seedlings grow rapidly and can reach 30 cm in a few weeks under ideal conditions (Panga, 2014).

Although it is less common, but still very effective, is by the propagation by semi-hardwood cuttings. The branches of the plant consisting of the current or last season's growth can be cut into 30 cm sections and planted in a moist potting mixture. This method is slower than seed propagation (cuttings take a few months to root) but is the preferred methods for ensuring new trees are true to form. As such, cuttings are a particularly common method of propagation for the rarer yellow-petals variety of the tree (Panga, 2014).

4.4 Medicinal Importance

Many scientists have reported that flowers and green leaves of the *Delonix regia* are useful as medicines (Coldin *et. al.*, 1985 and Koshimizur, 1988). The leaves are reported for their anti-microbial and anti-oxidant effect (Aly *et. al.*, 2011). It was reported that the plant of *D. regia* is used in many countries for the preparation of extracts having anti-fungal and anti-microbial activities (Sammour *et. al.*, 1992) and in one of the study done by Aqil and team (2003) it is also used as antibiotics. The parts of the plant including the petals are used in the traditional medicines. In the rural areas, water extracts prepared from the petals of *D. regia* are used in homemade remedies. The red colour of the petals is due to the presence of the anthocyanin contents, which is still not well studied with modern analytical techniques to determine their molecular structures and their possible applications, such as

natural pH indicators (Gupta *et. al.*, 1971; Banerjee and De, 2001 and Soltan *et. al.*, 2001). The tentative anthocyanin identification was made in 1971 on *D. regia* petals extracts, which were collected near Cairo (Saleh *et. al.*, 1976). The flowers of *D. regia* also contain carotenoides (Jungalwala and Chama, 1962), tannins, saponins, flavonoids, steroids, β-sitosterol and alkaloids (Parekh and Chanda, 2007). The seed consists of saponins and galactomannon (Jungalwala and Cama, 1962).

4.5 Determination of Extraction Yield of Plant Extract (% yield)

The initial weight of 30 gms of the dried petals of *Delonix regia* was taken in 100 ml of methanol. The percentage yield of 6.16 percent was obtained in the methanolic extract of petals of *D. regia,* whereas 6.68 percent in the methanolic extract of leaves of *D. regia*. The percentage yield of extracts of petals and leaves of *D. regia* in methanol is given in Table 4.1.

S.No.	Plant name	Weight of dried plant W_0 (gm)	Weight of empty petri plate W_1 (gm)	Weight of petri plate with plant extract W_2 (gm)	Percentage yield (%)
1.	*Delonix regia* petals	30 gm	46.700 gm	48.550 gm	6.16
2.	*Delonix regia* leaves	30 gm	48.700 gm	50.705 gm	6.68

Table No. 4.1: The percentage yield of methanolic extracts of *Delonix regia*. The extraction done by soaking dried plant material in methanol and the extract separation done using distilling apparatus.

4.6 Total Phenolics Estimation of Petals and Leaves Extracts

The total phenolic content found in the petals extract and leaves extract of *D. regia* were estimated spectrophotometrically using the Folin-Ceocalteu Reagent at 765 nm.

A calibration curve was drawn using Gallic Acid which was used as standard. The level of gallic acid in the methanolic extract of petals and leaves extract was measured respectively. The observed concentrations were multiplied with dilution factor. The results were reported as Gallic Acid Equivalent, (GAE) in mg/g of dry mass.

The gallic acid is a stable substance which is pure in nature and it is easily available. Since this assay measures all phenolics. The stability of gallic acid standard solutions was also tested and it shows that it loses less than 5% of their value over two weeks when refrigerated and kept tightly closed (Waterhouse, 1999 and 2009). The GAE for *D. regia* methanolic petals extract and leaves extract were estimated to be 2.67 GAE/g and 2.74 GAE/g respectfully. The standard calibration curve is shown in Figure 4.2.

4.7 Tannins Estimation of Petals and Leaves Extracts

The total tannins content was also estimated spectrophotometrically at 765 nm using Folin-Denis Reagent here tannic acid was used as standard. The total phenolic content was expressed as mg/g tannic acid equivalents per gram, (TAE) expressed in mg/g of dry mass using the following equation based on the calibration curve:

$$y = 0.002x + 0.98, R^2 = 0.979$$

The experiment was replicated thrice and average data recorded for quality assurance. The TAE for *D. regia* petals and leaves extract were estimated to be 1.042 TAE/g and 1.056 TAE/g respectfully. The standard calibration curve is shown in Figure 4.3.

4.8 Phyto-chemical Analysis of Petals Extracts and Leaves Extract of *D. regia*

Phyto-chemical analysis involves the qualitative analysis of herbal plant exracts. The preliminary qualitative tests have been attempted in *Delonix regia* petals and leaves respectively, to find out the presence or absence of certain bio active compounds. The chemical tests were carried out on the crude methanolic extract using standard procedures to identify the active constituents.

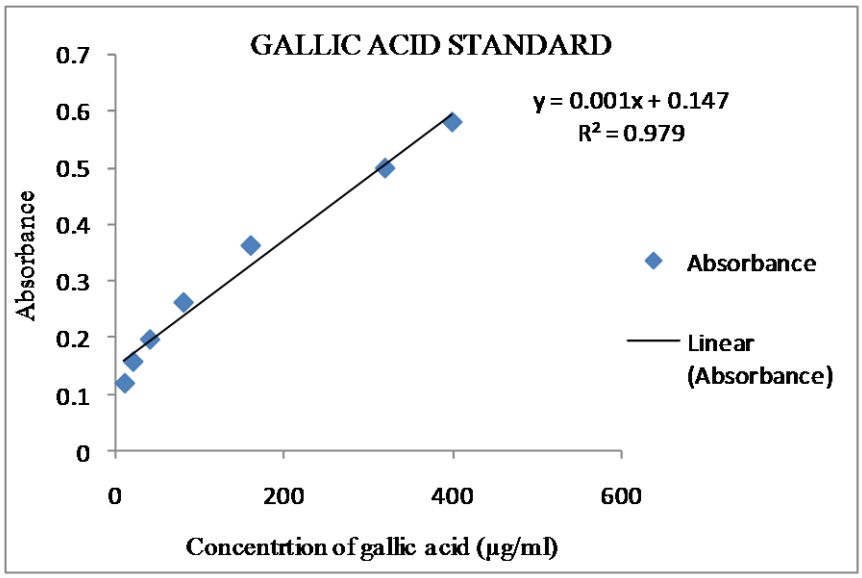

Figure No. 4.2: Calibration curve for gallic acid for determining the phenolic content.

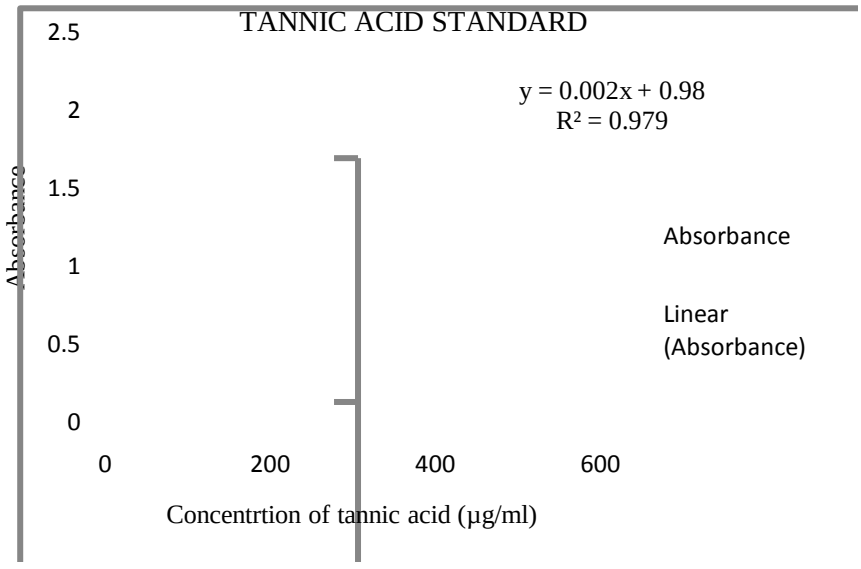

Figure No. 4.3: Calibration curve for tannic acid for determining the tannin content.

The crude methanolic extracts of petals and leaves of *D. regia* were evaluated qualitatively to analyze the presence of secondary metabolites. The secondary metabolites found in the crude methanolic extract of petals are alkaloids, flavonoids, phlobatanins, glycosides and tannins. Whereas, the secondary metabolites found in crude methanolic extract of leaves were found to be anthraquinones, flavonoids, glycosides, steroids, tannins and terpenoids. The *D. regia* crude methanolic extract of petals and leaves showed positive result to the different phyto-chemical tests indicating the presence a number of phyto-constituents. The results of qualitative phyto-chemical analysis are given in Table 4.2.

The presence of alkaloid was analyzed in methanolic extract of petals and leaves with Wagner's method. The presence of reddish brown colored precipitate indicates the presence of alkaloids. The absence of reddish brown colored precipitate in the methanolic leaves extract, indicating the absence of alkaloids in them.

The Borntrager's test was performed for the analysis of anthraquinones in the methanolic extract of petals and leaves. The formation of rose pink colour in plant extract confirmed the presence of anthraquinones. The methanolic petals extract when tested using this assay showed the absence of anthraquinones as no pink colour was formed, whereas the methanolic leaves extract when tested using the Borntrager's test confirmed the appearance of pink colour indicating the presence of anthraquinones in the methanolic extract of leaves.

The presence of flavonoids in the crude plant extract is determined quantitatively by the appearance of yellow colour. When the methanolic extract of petals and leaves of *D. regia* was evaluated using this test showed the appearance of yellow colour indicates the presence of flavonoids in both.

The presence of phlobatannins was evaluated qualitatively by adding 1% of aqueous HCl in boiled crude methanoilic extract of *D. regia* petals and leaves. The presence of red colour indicates a positive result. The crude methanolic extracts of *D. regia* petals showed the presence of red colour indicating the presence of phlobatannins while the methanolic extract of leaves did not show the red colour precipitates thus indicating

that phlobatannins are absent in the leaves extract.

The presence of glycosides in the *D. regia* methanolic leaves and petals extract was evaluated using the Fehling's test. The brick red precipitate formation indicates the presence of glycosides. *D. regia* leaves extract as well as petals extract showed the presence of brick red precipitate thus confirming the presence of glycosides in both crude extracts.

Similarly the presence of saponins in the plant extracts evaluated using a frothing test. The formation of froth confirmed the presence of saponins. The *D. regia* leaves extracts as well as petals extracts did not show the appearance of froth indicating the absence of saponins in both the extracts.

The *D. regia* crude methanolic leaves and petal extracts were also evaluated for the presence of steroids by using the Salkwoski test. The change of colour from violet to blue indicates a positive result. The crude methanolic petals extract did not show the change in colour indicating the absence of steroids while the crude methanolic leaves extracts showed the change of colour from violet to blue thus confirming the presence of steroids in the extract.

The crude leaves and petals extract were further tested for the presence of tannins by using ferric chloride test. The occurrence of blue black precipitate indicates the presence of tannins. The *D. regia* both methanolic leaves and petal extract showed the formation of blue black precipitate thus confirming the presence of tannins in both.

Similarly, Salkowski test was also performed to evaluate the presence of terpenoids in *D. regia* curde methanolic extract of leaves and petals. The formation of reddish brown colour indicates the presence of terpenoid. The petal extract did not show change in the colour indicating the absence of terpenoids where as the leaves extract confirmed the presence of terpenoids with the appearance of reddish brown colour.

S.No.	Active principle	Tests for Phytoconstituents	Petals Extract Result	Leaves Extract Result
1.	Alkaloids	Wagner's Test	+	-
2.	Anthraquinones	Borntrager's Test	-	+
3.	Flavonoids	Sodium Hydroxide (NaOH) Test	+	+
4.	Phlobatanins	Hydrocloric Acid (HCl) Test	+	-
5.	Glycosides	Fehling's Test	+	+
6.	Saponins	Frothing Test	-	-
7.	Steroids	Salkwoski Test	-	+
8.	Tannins	Ferric chloride ($FeCl_3$) Test	+	+
9.	Terpenoids	Salkwoski Test	-	+

Table No. 4.2: Phyto-chemical analysis of methanolic extract of petals and leaves of *D. regia*

4.9 Anti-oxidant Activities of Petals and Leaves Extract of *Delonix regia*

Anti-oxidant activity of methanolic petals and leaves extract of *D. regia* was determined *in vitro* by using a number of assays were super oxide scavenging activity by alkaline DMSO method, DPPH free radical scavenging activity, nitric oxide free radical scavenging activity, H_2O_2 radical scavenging activity and total anti-oxidant capacity method.

4.9.1 Scavenging of Superoxide Radical with the Alkaline DMSO (Dimethyl Sulfoxide) Method

The superoxide radical scavenging assay, were studied in crude methanolic petals and leaves extract of *D. regia* at different concentrations ranging from 1.95 to 1000 µg/ml and absorbance measured at the wavelength of 560 nm. The results are given as percentage inhibition values of the extract. The increase in percentage showed stronger inhibition and highest scavenging activity of the petals and leaves extract.

In the methanolic petal extract the percentage inhibition values were found to range between 74.32 ± 0.115 and 2.00 ± 0.484 percent, at the concentration of 1000 and 1.95 µg/ml respectively. While in the methanolic leaves extract the percent inhibition values were found to range between 77.90 ± 0.074 and 5.26 ± 0.453 percent, at the concentration of 1000 and 1.95 µg/ml respectively, whereas the percentage inhibition values of BHT were found to be 63.52 ± 0.020 and 6.63 ± 0.229 percent, at the concentration of 1000 and 1.95 µg/ml respectively. The percentage inhibition values of *D. regia* methanolic extracts of petals and leaves extract along with standard (BHT) were given in Table 4.3, 4.4 and 4.5 and Figure 4.4.

The crude methanolic extract of both *D. regia* leaves and petals scavenges super oxide radical and thus inhibits formazan formation. In Table 4.3 and 4.4 it is illustrated that increase in scavenging of superoxide radicals in dose dependent manner due to the scavenging ability of the *D. regia* methanolic extract. The IC_{50} value of *D. regia* petals extract was found to be 86.33 ± 2.482 µg/ml, while in *D. regia* leaves extract was 179.66 ± 2.309 µg/ml, whereas in butylated hydroxytoluene the IC_{50} value was 792.49 ± 1.16 µg/ml.

4.9.2 Nitric Oxide Free Radical Scavenging Activity

The crude methanolic petals and leaves extract of *D. regia* were evaluated using the nitric oxide free radical scavenging activity. The standard used for the study was butylated hydroxytoluene (BHT). The methanolic extract of petals and

leaves of *D. regia* showed significant scavenging activity.

The percentage inhibition values were found to be 88.49 ± 1.55 and 3.09 ± 1.069 percent respectively, at the concentration of 1000 and 1.95 µg/ml. While in the methanolic leaves extract the percent inhibition values were found to be 79.58 ± 0.211 and 1.50 ± 0.401 at the concentration of 1000 and 1.95 µg/ml respectively, whereas the percentage inhibition values of BHT were found to be 56.44 ± 0.113 and 1.90 ± 0.380 percent at the concentration of 1000 and 1.95 µg/ml respectively. The percentage inhibition values of *D. regia* methanolic extracts of petals, leaves extract along with standard (BHT) were shown in Table 4.6, 4.7 and 4.8 and Figure 4.5.

The high percentage inhibition indicates high scavenging activity of the plant extract. The IC_{50} value of *D. regia* petals extract was 89.03 ± 0.85 µg/ml and *D. regia* leaves extract was 108.4 ± 0.3 µg/ml. Whereas IC_{50} value of BHT was 364.60 ± 3.51 µg/ml.

4.9.3 Scavenging of Radical with the H_2O_2 (Hydrogen peroxide) Method

The hydrogen peroxide is not a strong oxidizing agent. It can cause inactivation of some enzymes directly, by oxidation of the thiol (-SH) groups. It can easily cross cell membrane rapidly. Once reached inside the cell, H_2O_2 can possibly reacts with Fe^{2+} and possibly Cu^{2+} to form hydroxyl radical. The formation of hydroxyl radical is the initial step of the formation of many toxic effects (Miller *et. al.*, 1993).

It is therefore very important and necessary for the cells to control the production of hydrogen peroxide which was built up *in vivo*. The scavenging of H_2O_2 attributes to their phenolic content which donate electrons to H_2O_2, thus was neutralizing it to water (Halliwell and Gutteridge, 1985).

The ability of the extract to effectively scavenge hydrogen peroxide were determined according to the method done by Ruch *et. al.*, (1989) where they were

compared with BHT. The *D. regia* methanolic extracts were capable of scavenging hydrogen peroxide in a concentration dependent manner.

The methanolic petals extracts exhibited 74.25 ± 0.398 and 1.06 ± 0.578 percent inhibition respectively, whereas the leaves extracts exhibited 68.84 ± 0.45 and 1.15 ± 0.381 percent inhibition respectively, at the concentration of 1000 µg/ml and 1.95 µg/ml by hydrogen peroxide scavenging activity. On the other hand, at the same concentration butylated hydroxy toluene exhibited 77.03 ± 0.128 and 4.14 ± 0.128 percent inhibition respectively. The percentage inhibition values of *D. regia* methanolic extracts of petals and leaves extract along with standard (BHT) were shown in Table 4.9, 4.10 and 4.11 and Figure 4.6. The IC_{50} value of *D. regia* petals extract was found to be 140.33 ± 4.99 µg/ml while the IC_{50} value of *D. regia* leaves extract was found to be 326.43 ± 5.773 µg/ml whereas the IC_{50} value of BHT was found to be 26.16 ± 0.351 µg/ml.

4.9.4 Anti-oxidant Activity by DPPH (2, 2 – Diphenyl – 1- Picryl Hydrazyl) Radical Scavenging Assay

The DPPH radical scavenging assay showed the ability of the extracts and the standard (BHT) to scavenge DPPH free radicals. The DPPH radical exists naturally in deep violet colour but when reacts with anti-oxidant it turn into a yellow coloured diphenyl picryl hydrazine. The degree of discoloration indicates the radical-scavenging potential of the anti-oxidant (Tirzitis and Bartosz, 2010).

The methanolic petals extracts exhibited 70.75 ± 0.294 and 0.12 ± 0.170 percent inhibition, while the methanolic leaves extracts exhibited 64.77 ± 0.456 and 1.03 ± 0.222 percent inhibition, at the concentration of 1000 µg/ml and 1.95 µg/ml respectively, whereas the percentage inhibition values of BHT were found to be 73.03 ± 0.128 and 12.59 ± 0.128 percent, at the concentration of 1000 µg/ml and 1.95 µg/ml respectively. The DPPH radical scavenging activity values of the *D. regia* methanolic petals and leaves extracts along with standard (BHT) were shown in Table 4.12, 4.13 and 4.14 and Figure 4.7. The high percentage inhibition indicates high scavenging activity of the plant extract. The IC_{50} value of *D. regia*

petals extract was found to be 155.5 ± 5.54 µg/ml, while *D. regia* leaves extract was found to be 332.2 ± 3.983 µg/ml. Whereas IC_{50} value of butylated hydroxytoluene was 43.40 ± 1.307 µg/ml.

4.9.5 Total Anti-oxidant Capacity by Phosphomolybdenum Method

The total anti-oxidant capacity of the methanolic crude plant extracts and standard (BHT) were determined by using the method of phosphomolybdenum. The higher absorbance value indicates the greater anti-oxidant activity. The total anti-oxidant capacity of plant extracts were measured at 695nm, spectrophotometrically using total anti-oxidant activity by phosphomolybdenum method. This method is based on the reduction of Mo (IV) to Mo (V) by the test sample and the formation of green phosphate/Mo (V) compounds (Abbasi *et. al.*, 2010). A high absorbance value of the sample indicates its strong anti-oxidant activity. The total anti-oxidant capacity may be contributed due to their chemical composition and phenolic acid content.

The methanolic petals extracts exhibited 59.92 ± 0.894 and 0.32 ± 1.132 percent inhibition, whereas leaves extracts exhibited 50.37 ± 0.189 and 0.25 ± 0.109 percent inhibition, at the concentration of 1000 µg/ml and 1.95 µg/ml respectively. The percentage inhibition values of standard (BHT) were found to be 77.12 ± 0.322 and 20.10 ± 0.207 percent, at the concentration of 1000 µg/ml and 1.95 µg/ml respectively. The values of the methanolic extracts along with standard (BHT) of total anti-oxidant capacity by phosphomolybdenum method were shown in Table 4.15, 4.16 and 4.17 and Figure 4.8. The high percentage inhibition indicates high scavenging activity of the plant extract. The IC_{50} value of *D. regia* petals extract was found to be 250 ± 0.00 µg/ml and *D. regia* leaves extract was found to be 976.84 ± 13.140 µg/ml, whereas the IC_{50} value of butylated hydroxytoluene was 124.25 ± 3.04 µg/ml.

4.10 IC_{50} Value of Different Anti-oxidant Activity

The IC_{50} values of the methanolic extracts were calculated based on the results of different anti-oxidant assay conducted by DPPH, Alkaline DMSO, Nitric oxide scavenging assay, total anti-oxidant assay and hydrogen peroxide method. The results are given below in Table 4.18.

S.No.	Plant conc. (µg/ml)	*Delonix regia* (Petals) (Absorbance)	Percent Inhibition (%)
1.	1000	0.444 ± 0.002	74.32 ± 0.115
2.	500	0.371 ± 0.001	68.82 ± 0.494
3.	250	0.310 ± 0.000	63.30 ± 0.068
4.	125	0.277 ± 0.001	58.84 ± 0.148
5.	62.5	0.209 ± 0.000	45.54 ± 0.149
6.	31.25	0.198 ± 0.000	42.52 ± 0.167
7.	15.625	0.145 ± 0.001	21.37 ± 0.542
8	7.8125	0.130 ± 0.001	12.30 ± 0.674
9.	3.906	0.119 ± 0.001	4.72 ± 0.914
10.	1.95	0.116 ± 0.000	2.00 ± 0.484

Table No. 4.3: The different concentrations of extracts used from 1000 to 1.95 µg/ml. The data represent the percentage alkaline DMSO inhibition. Values are expressed as mean ± SD (n=3).

S.No.	Plant conc. (µg/ml)	*Delonix regia* (Leaves) (Absorbance)	Percent Inhibition (%)
1.	1000	0.516 ± 0.001	77.90 ± 0.074
2.	500	0.355 ± 0.001	67.88 ± 0.090
3.	250	0.255 ± 0.000	55.41 ± 0.100
4.	125	0.211 ± 0.000	46.05 ± 0.147
5.	62.5	0.198 ± 0.001	42.42 ± 0.290
6.	31.25	0.151 ± 0.001	24.50 ± 0.500
7.	15.625	0.145 ± 0.001	21.73 ± 0.823
8.	7.8125	0.139 ± 0.000	18.37 ± 0.338
9.	3.906	0.132 ± 0.000	14.06 ± 0.374
10.	1.95	0.120 ± 0.000	5.26 ± 0.453

Table No. 4.4: The different concentrations of extracts used from 1000 to1.95 µg/ml. The data represent the percentage alkaline DMSO inhibition. Values are expressed as mean ± SD (n=3).

S.No.	Plant conc. (µg/ml)	Butylated Hydroxytoluene (Absorbance)	Percent Inhibition (%)
1.	1000	1.041 ± 0.000	63.52 ± 0.020
2.	500	0.549 ± 0.000	30.82 ± 0.072
3.	250	0.532 ± 0.000	28.61 ± 0.077
4.	125	0.526 ± 0.001	27.75 ± 0.137
5.	62.5	0.488 ± 0.001	22.13 ± 0.159
6.	31.25	0.479 ± 0.001	20.66 ± 0.165
7.	15.625	0.461 ± 0.001	17.62 ± 0.206
8.	7.8125	0.435 ± 0.000	12.71 ± 0.115
9.	3.906	0.422 ± 0.001	9.95 ± 0.213
10.	1.95	0.407 ± 0.001	6.63 ± 0.229

Table No. 4.5: The different concentrations of extracts used from 1000 to1.95 µg/ml. Butylated hydroxytoluene was taken as standard. The data represent the percentage alkaline DMSO inhibition. Values are expressed as mean ± SD (n=3).

S. No.	Plant conc. (µg/ml)	*Delonix regia* (Petals) (Absorbance)	Percent Inhibition (%)
1.	1000	0.043 ± 0.005	88.49 ± 1.55
2.	500	0.081 ± 0.001	78.49 ± 0.237
3.	250	0.111 ± 0.001	70.53 ± 0.305
4.	125	0.150 ± 0.001	60.00 ± 0.348
5.	62.5	0.216 ± 0.001	42.65 ± 0.279
6.	31.25	0.242 ± 0.001	35.75 ± 0.282
7.	15.62	0.277 ± 0.001	26.28 ± 0.268
8.	7.812	0.320 ± 0.000	14.86 ± 0.246
9.	3.906	0.341 ± 0.001	9.46 ± 0.160
10.	1.95	0.365 ± 0.003	3.09 ± 1.069

Table No. 4.6: The different concentrations of extracts used from 1000 to 1.95 µg/ml. The data represent the percentage nitric oxide inhibition. Values are expressed as mean ± SD (n=3).

S. No.	Plant conc. (µg/ml)	*Delonix regia* (Leaves) (Absorbance)	Percent Inhibition (%)
1.	1000	0.077 ± 0.001	79.58 ± 0.211
2.	500	0.100 ± 0.01	73.47 ± 2.722
3.	250	0.142 ± 0.002	62.33 ± 0.630
4.	125	0.176 ± 0.000	53.22 ± 0.077
5.	62.5	0.222 ± 0.001	40.93 ± 0.253
6.	31.25	0.252 ± 0.000	33.15 ± 0.177
7.	15.625	0.281 ± 0.001	25.46 ± 0.067
8.	7.8125	0.322 ± 0.001	14.85 ± 0.039
9.	3.906	0.351 ± 0.001	6.89 ± 0.512
10.	1.95	0.371 ± 0.000	1.50 ± 0.401

Table No. 4.7: The different concentrations of extracts used from 1000 to 1.95 µg/ml. The data represent the percentage nitric oxide inhibition. Values are expressed as mean ± SD (n=3).

S.No.	Plant conc. (µg/ml)	Butylated Hydroxytoluene (Absorbance)	Percent Inhibition (%)
1.	1000	0.011 ± 0.000	56.44 ± 0.113
2.	500	0.121 ± 0.001	53.63 ± 0.4997
3.	250	0.138 ± 0.000	46.87 ± 0.111
4.	125	0.144 ± 0.001	44.82 ± 0.332
5.	62.5	0.151 ± 0.001	42.14 ± 0.604
6.	31.25	0.176 ± 0.001	32.56 ± 0.641
7.	15.625	0.203 ± 0.001	22.22 ± 0.681
8.	7.8125	0.212 ± 0.001	18.77 ± 0.352
9.	3.906	0.222 ± 0.001	14.94 ± 0.358
10.	1.95	0.257 ± 0.001	1.90 ± 0.380

Table No. 4.8 The different concentrations of standard used from 1000 to 1.95 µg/ml. The standard used was butylated hydroxytoluene. The data represent the percentage nitric oxide inhibition. Values are expressed as mean ± SD (n=3).

S.No.	Plant conc. (µg/ml)	*Delonix regia* (Petals) (Absorbance)	Percent Inhibition (%)
1	1000	0.072 ± 0.001	74.25 ± 0.398
2	500	0.111 ± 0.001	60.37 ± 0.355
3	250	0.122 ± 0.002	56.58 ± 0.528
4	125	0.143 ± 0.001	49.11 ± 0.251
5	62.5	0.156 ± 0.002	44.36 ± 0.452
6	31.25	0.167 ± 0.001	40.56 ± 0.406
7	15.625	0.206 ± 0.001	26.57 ± 0.654
8	7.812	0.211 ± 0.001	24.79 ± 0.660
9	3.906	0.221 ± 0.001	21.35 ± 0.451
10	1.95	0.278 ± 0.001	1.06 ± 0.578

Table No. 4.9: The different concentrations of extracts used from 1000 to 1.95 µg/ml. The data represent the percentage hydrogen peroxide inhibition. Values are expressed as mean ± SD (n=3).

S.No.	Plant conc. (µg/ml)	*Delonix regia* (Leaves) (Absorbance)	Percent Inhibition (%)
1.	1000	0.081 ± 0.001	68.84 ± 0.457
2.	500	0.101 ± 0.001	61.15 ± 0.336
3.	250	0.143 ± 0.001	44.87 ± 0.555
4.	125	0.166 ± 0.001	36.15 ± 0.551
5.	62.5	0.175 ± 0.001	32.43 ± 0.513
6.	31.25	0.186 ± 0.001	28.33 ± 0.797
7.	15.625	0.198 ± 0.001	23.84 ± 0.348
8.	7.8125	0.212 ± 0.001	18.46 ± 0.604
9.	3.906	0.221 ± 0.001	15.00 ± 0.615
10.	1.95	0.257 ± 0.001	1.15 ± 0.381

Table No. 4.10: The different concentrations of extracts used from 1000 to 1.95 µg/ml. The data represent the percentage hydrogen peroxide inhibition. Values are expressed as mean ± SD (n=3).

S.No.	Plant conc. (µg/ml)	Butylated hydroxytoluene (Absorbance)	Percent Inhibition (%)
1.	1000	0.103 ± 0.005	77.03 ± 0.128
2.	500	0.119 ± 0.000	73.48 ± 0.128
3.	250	0.136 ± 0.000	69.70 ± 0.128
4.	125	0.167 ± 0.001	62.88 ± 0.222
5.	62.5	0.200 ± 0.001	55.55 ± 0.222
6.	31.25	0.217 ± 0.001	51.77 ± 0.222
7.	15.625	0.241 ± 0.000	46.37 ± 0.128
8.	7.8125	0.313 ± 0.001	30.37 ± 0.339
9.	3.906	0.380 ± 0.001	15.55 ± 0.222
10.	1.95	0.431 ± 0.000	4.14 ± 0.128

Table No. 4.11: The different concentrations of standards used from 1000 to 1.95 µg/ml. The standard used was butylated hydroxytoluene. The data represent the percentage hydrogen peroxide inhibition. Values are expressed as mean ± SD (n=3).

S.No.	Plant conc. (µg/ml)	*Delonix regia* (Petals) (Absorbance)	Percent Inhibition (%)
1.	1000	0.081 ± 0.001	70.75 ± 0.294
2.	500	0.111 ± 0.000	59.68 ± 0.170
3.	250	0.131 ± 0.000	52.70 ± 0.00
4.	125	0.140 ± 0.000	49.21 ± 0.170
5.	62.5	0.161 ± 0.001	41.87 ± 0.294
6.	31.25	0.181 ± 0.001	34.53 ± 0.450
7.	15.625	0.220 ± 0.000	20.33 ± 0.170
8.	7.8125	0.241 ± 0.001	12.99 ± 0.294
9.	3.906	0.251 ± 0.001	9.38 ± 0.294
10.	1.95	0.276 ± 0.000	0.12 ± 0.170

Table No. 4.12: The different concentrations of extracts used from 1000 to 1.95 µg/ml. The data represent the percentage DPPH inhibition values. Values are expressed as mean ± SD (n=3).

S.No.	Plant conc. (µg/ml)	*Delonix regia* (Leaves) (Absorbance)	Percent Inhibition (%)
1.	1000	0.091 ± 0.001	64.77 ± 0.456
2.	500	0.113 ± 0.000	56.12 ± 0.125
3.	250	0.137 ± 0.000	46.83 ± 0.104
4.	125	0.152 ± 0.001	41.16 ± 0.280
5.	62.5	0.168 ± 0.001	34.96 ± 0.271
6.	31.25	0.182 ± 0.000	29.29 ± 0.199
7.	15.625	0.203 ± 0.001	21.41 ± 0.425
8.	7.8125	0.232 ± 0.001	10.19 ± 0.235
9.	3.906	0.251 ± 0.001	2.83 ± 0.584
10.	1.95	0.255 ± 0.000	1.03 ± 0.222

Table No. 4.13: The different concentrations of extracts used from 1000 to 1.95 µg/ml. The data represent the percentage DPPH inhibition values. Values are expressed as mean ± SD (n=3)

S.No.	Plant conc. (µg/ml)	Butylated Hydroxytoluene (Absorbance)	Percent Inhibition (%)
1.	1000	0.121 ± 0.000	73.03 ± 0.128
2.	500	0.149 ± 0.000	66.81 ± 0.128
3.	250	0.194 ± 0.001	56.88 ± 0.222
4.	125	0.201 ± 0.000	55.25 ± 0.128
5.	62.5	0.213 ± 0.002	52.59 ± 0.462
6.	31.25	0.232 ± 0.000	48.44 ± 0.000
7.	15.625	0.248 ± 0.001	44.88 ± 0.222
8.	7.8125	0.287 ± 0.000	36.14 ± 0.128
9.	3.906	0.331 ± 0.000	26.29 ± 0.128
10.	1.95	0.393 ± 0.000	12.59 ± 0.128

Table No. 4.14: The different concentrations of standard used from 1000 to 1.95 µg/ml. The standard used was butylated hydroxytoluene. The data represent the percentage DPPH inhibition values. Values are expressed as mean ± SD (n=3).

S.No.	Plant conc. (µg/ml)	*Delonix regia* (Petals) (Absorbance)	Percent Inhibition (%)
1.	1000	0.040 ± 0.000	59.92 ± 0.894
2.	500	0.045 ± 0.001	54.96 ± 0.941
3.	250	0.050 ± 0.001	50.00 ± 0.000
4.	125	0.055 ± 0.000	45.02 ± 0.1.039
5.	62.5	0.060 ± 0.000	40.05 ± 1.089
6.	31.25	0.070 ± 0.000	30.12 ± 1.191
7.	15.625	0.076 ± 0.001	24.16 ± 1.253
8.	7.8125	0.087 ± 0.000	13.12 ± 1.368
9.	3.906	0.098 ± 0.000	2.30 ± 1.50
10.	1.95	0.100 ± 0.001	0.32 ± 1.132

Table No. 4.15: The different concentrations of extracts used from 1000 to 1.95 µg/ml. The data represent the percentage inhibition values. Values are expressed as mean ± SD (n=3).

S.No.	Plant conc. (µg/ml)	*Delonix regia* (Leaves) (Absorbance)	Percent Inhibition (%)
1.	1000	0.262 ± 0.001	50.37 ± 0.189
2.	500	0.298 ± 0.001	43.56 ± 0.189
3.	250	0.356 ± 0.001	32.57 ± 0.189
4.	125	0.421 ± 0.000	20.13 ± 0.109
5.	62.5	0.455 ± 0.000	13.76 ± 0.109
6.	31.25	0.480 ± 0.000	9.02 ± 0.109
7.	15.625	0.501 ± 0.001	5.11 ± 0.189
8.	7.8125	0.510 ± 0.000	3.28 ± 0.109
9.	3.906	0.520 ± 0.000	1.38 ± 0.109
10.	1.95	0.526 ± 0.000	0.25 ± 0.109

Table No. 4.16: The different concentrations of extracts used from 1000 to 1.95 µg/ml. The data represent the percentage inhibition values by TAC method. Values are expressed as mean ± SD (n=3).

S.No.	Plant conc. (µg/ml)	Butylated HydroxyTotuene (Absorbance)	Percent Inhibition (%)
1.	1000	0.099 ± 0.001	77.12 ± 0.322
2.	500	0.143 ± 0.001	66.92 ± 0.290
3.	250	0.176 ± 0.001	59.40 ± 0.365
4.	125	0.217 ± 0.001	50.03 ± 0.175
5.	62.5	0.234 ± 0.000	46.05 ± 0.061
6.	31.25	0.269 ± 0.000	38.06 ± 0.082
7.	15.625	0.300 ± 0.000	30.92 ± 0.091
8.	7.812	0.319 ± 0.001	26.47 ± 0.233
9.	3.906	0.337 ± 0.001	22.25 ± 0.347
10.	1.95	0.347 ± 0.001	20.10 ± 0.207

Table No. 4.17: The different concentrations of standard used from 1000 to 1.95 µg/ml. The standard used was butylated hydroxytoluene. The data represent the percentage inhibition values. Values are expressed as mean ± SD (n=3).

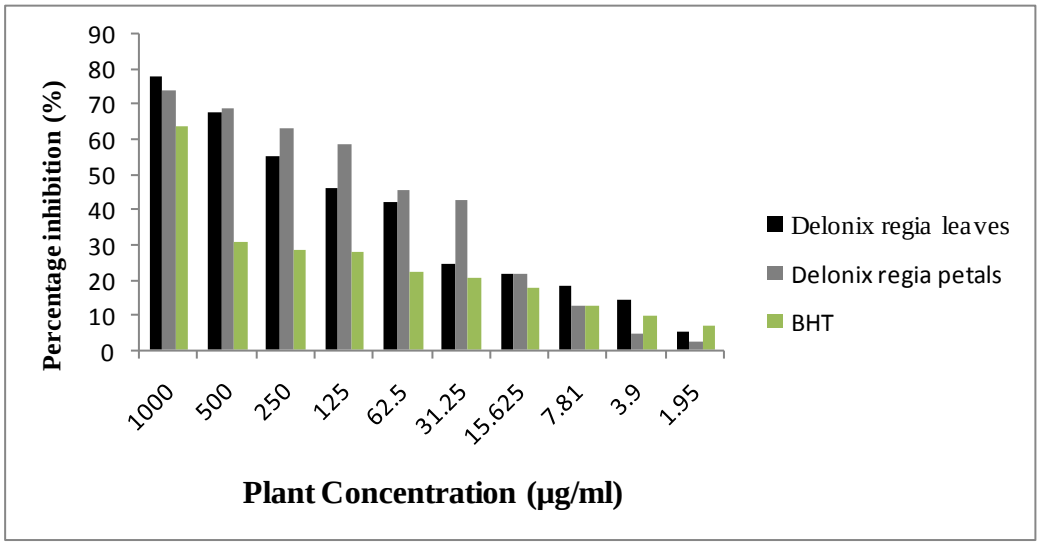

Figure No. 4.4: Graphical representation of percent inhibition of methanolic extract of leaves and petals of *Delonix regia* and butylated hydroxy toluene (BHT) as standard by using Alkaline DMSO method.

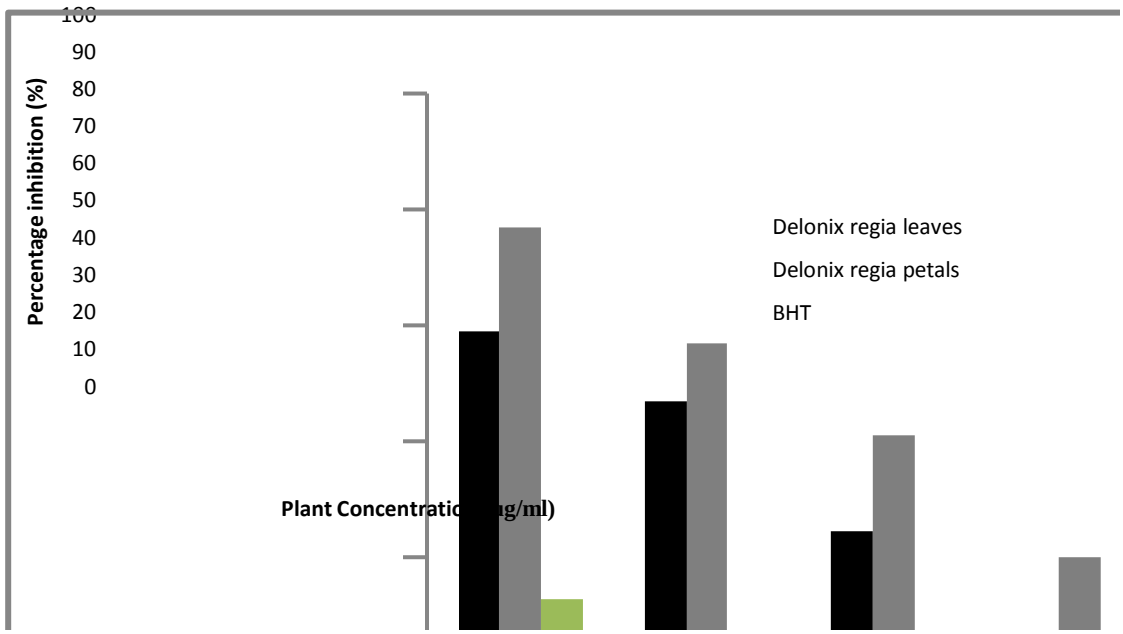

Figure No. 4.5: Graphical representation of percent inhibition of methanolic extract of leaves and petals of *Delonix regia* and Butylated hydroxy toluene (BHT) as standard by using nitric oxide radical scavenging activity**.**

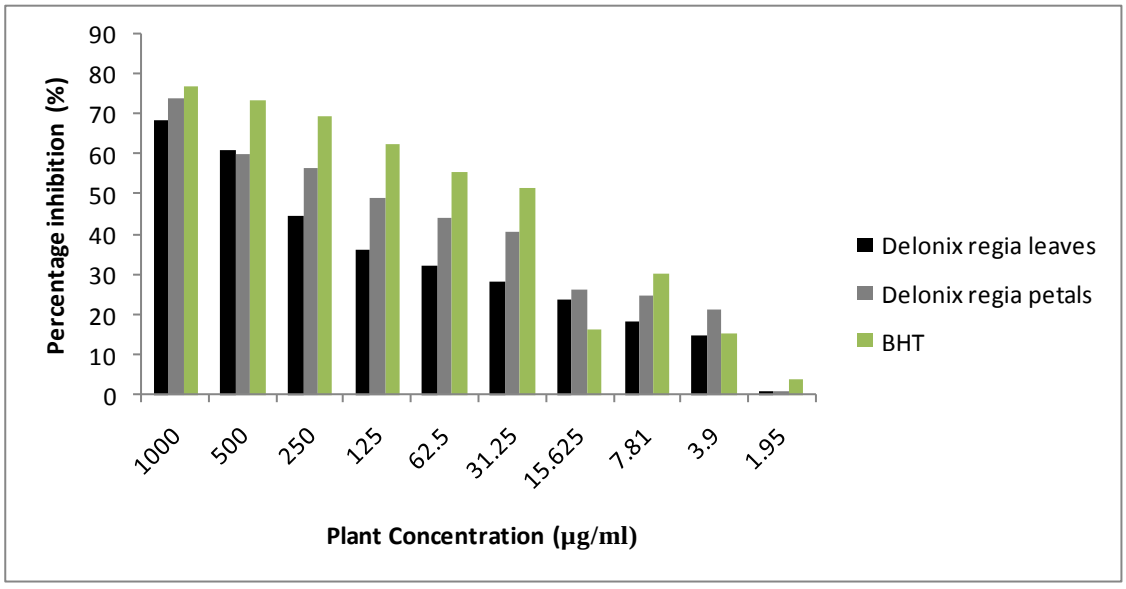

Figure No. 4.6: Graphical representation of percent inhibition of methanolic extract of leaves and petals of *Delonix regia* and Butylated hydroxy toluene (BHT) as standard by using hydrogen peroxide scavenging method**.**

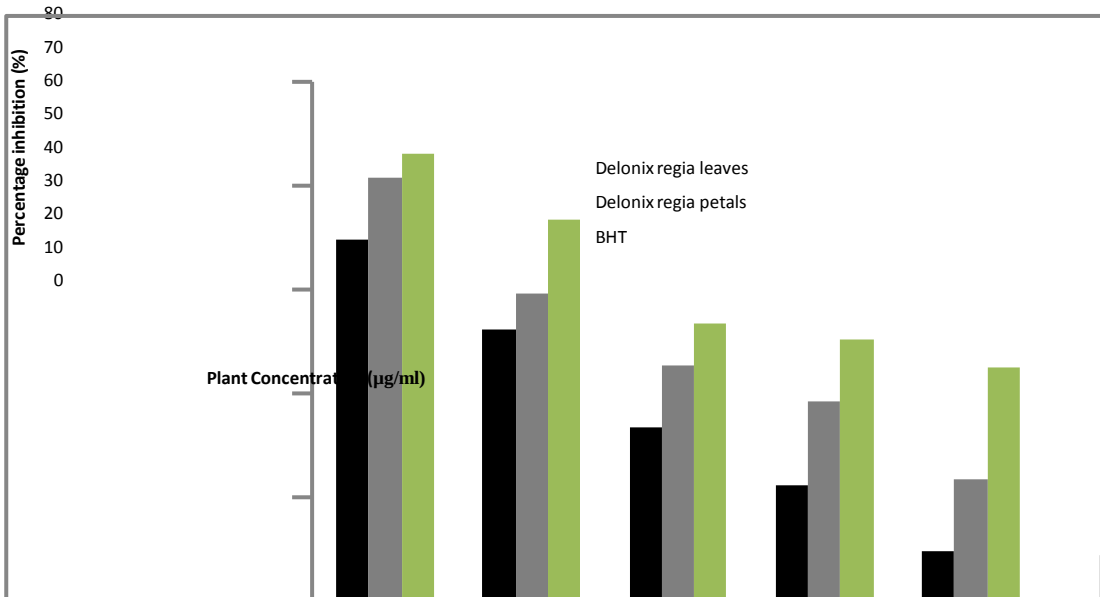

Figure No. 4.7: Graphical representation of percent inhibition of methanolic extract of leaves and petals of *Delonix regia* and Butylated hydroxy toluene (BHT) as standard by using DPPH radical scavenging activity**.**

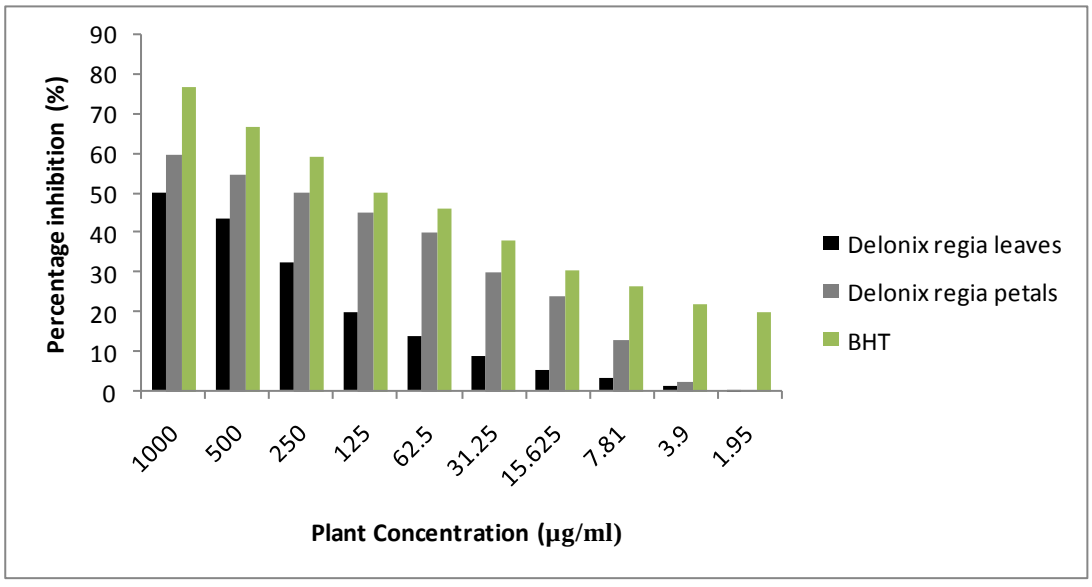

Figure No. 4.8: Graphical representation of percent inhibition of methanolic extract of leaves and petals of *Delonix regia* and Butylated hydroxy toluene (BHT) as standard by using total anti-oxidant capacity.

S. No.	Test Performed	IC_{50} value for Petals Extract	IC_{50} value for Leaves Extract	Butylated Hydroxytoluene
1.	Alkaline DMSO Method	86.33 ± 2.48	179.66 ± 2.30*	792.49 ± 1.16
2.	DPPH Method	155.5 ± 5.54	332.2 ± 3.983*	43.40 ± 1.307
3.	H_2O_2 Method	140.33 ± 4.99	326.43 ± 5.773	26.166 ± 0.351
4.	Nitric Oxide Method	89.03 ± 0.85*	108.40 ± 0.30*	364.60 ± 3.510
5.	Total Anti-oxidant Capacity Method	250.00 ± 0.00	976.84 ± 13.149	124.25 ± 3.040

Table No. 4.18: IC_{50} value of different anti-oxidant activity of methanolic petals and leaves extract of *D. regia*. The standard used was butylated hydroxytoluene (BHT). Unit for IC_{50} for all the activities are µg/ml. Data are expressed as mean ± SD (n=3) (*P value : < 0.0001).

4.11 Thin Layer Chromatography

Thin layer chromatography (TLC) is a sophisticated method and a type of planar chromatography used in the present study to identify the components in the *Delonix regia* methanolic petals and leaves extract such as alkaloids, phenols, flavonoids etc. The separation depends on the relative affinity of compounds towards stationary and mobile phase. The plant compounds travel under the influence of mobile phase (driven by capillary action) over to the surface of the stationary phase. The compound with higher affinity travels slowly in stationary phase while others travel faster. All the silica gel glass plates contained a reference spot along with the *Delonix regia* methanolic petals and leaves extract. The gallic acid and tannic acids were used as reference. The silica gel glass plates were developed in an iodine chamber in the presence of iodine fumes. Yellow to purplish pink color spots or rockets were observed. The R_f of the unknown compound is compared with R_f of the known compound (gallic acid and tannic acid). The R_f is the retention factor, indicating how far the compound has travelled on the silica gel plates.

Thin layer chromatogram was prepared by using 2 µg/ml of *Delonix regia* methanolic petals and leaves extract and standard compounds on silica gel plates. The R_f values of the extract and standard were observed, calculated and compared. It was found that the chromatogram had been showing bands at the similar distances as that of the band of tannic acid and gallic acid and their R_f values calculated were similar to standards.

The R_f value of methanolic petals and leaves extract was observed to be 0.77 and 0.80 respectively. The R_f value of gallic acid and tannic acid are 0.82 and 0.97.Thus, it indicates the presence of phenolics in the *D. regia* methanolic petals and leaves extract. Apart from these, other bands were also seen indicating the presence of other compounds as well. The results are shown in Figure 4.9.

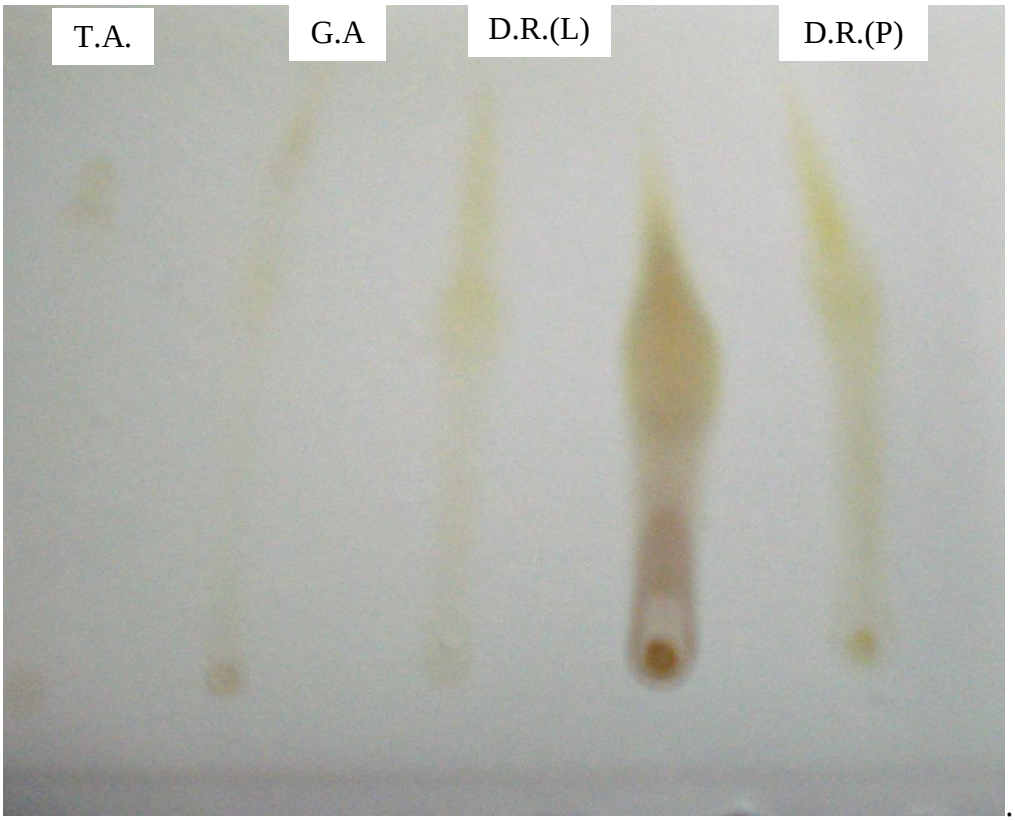

Figure No. 4.9: Tannic acid (T.A.), Gallic acid (G.A.), *D. regia* Flower (D.R. (L)) and *D. regia* Petals (D.R. (P))

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