CHAPTER – 8

ROSA INDICA

8.1 Introduction

Rosa indica belongs to the family of Rosaceae (Manjari *et. al.*, 2011). *Rosa indica* is commonly called as Rose in English language. It is referred to as Gulab in hindi and Gul-e-surkh in urdu languages. It is known for various pharmacological activities, and the presence of colored pigments and chemical constituents like flavonoids. It is also valued for their culinary, medicinal, cosmetic and aromatic properties.

8.2 Description of the Plant

The plant of *R. indica* is a yearly flowering plant and easily available in India and also throughout the world in high quantity and present in almost every garden to enhance the beauty of gardens. The plant is erect, perennial thorny shrub, containing essential oils (1%), other characteristic components of rose oil are acyclic mono-terpene alcohols, geraniol (up to 75%), citronellol (20%) and nerol (20%), and long-chain hydrocarbons like non-adecane or heneicosane (up to 10%). Peter Osbeck, a pupil of the great Swedish botanist Linnaeus, discovered *Rosa indica* in 1750. Graham Thomas was uncertain of this rose's origin and he stated that it is either a sport or a rose that is derived from an ancient hybrid of *Rosa chinensis*. *Rosa chinensis sanguinea*, Bengal Crimson, is a single China rose, whose colour varies from light to dark crimson. In several Chinese literatures, the rose is called Bengal Roses because they reached Europe via Bengal (Haynes, 2012).

Roses are one of the world’s most popular ornamental plants for a long time (Mishra *et. al.*, 2011). They are grown worldwide as cut flowers and potted plants and in home gardens. The flowers vary greatly in size, shape and colour. There are more than 20000 commercial cultivars of rose. They are regarded as the most important commercial crops, there are 200 wild species in Rosa (Razavizadeh and Ehsanpour, 2008 and Farahani and Sheker, 2012).

Roses can be propagated by seeds, cutting, layering and grafting. Seed propagation often

Figure 8.1: *Rosa indica*

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliolpsida
Order	:	Rosales
Family	:	Rosaceae
Genus	:	*Rosa*
Species	:	*indica*

results in variation while other methods of rose propagation are low and time consuming. So, there is need to introduce efficient methods for faster propagation of roses (Shabbir *et. al.*, 2009). The tissue culture system in roses has been established (Hsia and Korban, 1996; Kintzios *et. al.*, 1999, Ibrahim and Debergh, 2001; Kim *et. al.*, 2003; Rout *et. al.*, 2006; Hameed *et. al.*, 2006; Drefahl *et. al.*, 2007 and Previati *et. al.*, 2008). Recently, *in vitro* flower induction in roses was demonstrated by a number of researches (Wang *et. al.*, 2002 and Vu *et. al.*, 2006).

8.3 Ethno-botanical Importance

The rose commonly known as the Queen of Flowers is known as the beautiful flower of immense horticultural importance. Rose (*Rosa indica* L.) is used in religious rituals, medicines and social events (Krussmann, 1981). One of the primary problems with the roses is their susceptibility to diseases like black spot (*Diplocarpon rosae*), powdery mildew (*Sphaerotheca pannosa)*, bacterial blight, etc (Horst, 1983).

The leaves, stem, and flowers of *Rosa indica* have bacteriocidal effects on pathogenic micro-organisms. The tea prepared by brewing of leaves and petals of *R. indica* are known to lessen fever and common cold, also acts as a diuretic and uses to remove the toxins from the body. It is also known to relieve chest and bronchial congestion. Tea is also proven to heel the sore throat and also stops the runny nose. Rose water is also of great importance, it has been used by local practitioners since long time. Due to the nutritional value of rose hip, it is added in cooking for the enhancement of nutrition, and also when added it gives beautiful colour as well as flavour too. Oil extracted from rose is used in various skin problems, and also helps to moisturise the skin, makes it smooth and relieve skin irritation (Manjari *et. al.*, 2011).

Koday and his coworkers (2010) conducted anti-bacterial test on the extract of petals of *R. indica* and found strong activity against bacterial strains. In yet another study carried out done by Manjri and colleagues (2011) showed that the leaves of *R. indica* extracts have also shown significant results for their anti-bacterial activity.

8.4 Determination of Extraction Yield of Petals Extract (% yield)

The initial weight of 30 gm of the dried petals was taken in 100 ml of methanol. In methanolic extracts the percentage yield in the petals of *Rosa indica* was 6.75 percent.

The percentage yield of extracts of petals of *R. indica* in methanol is given below in Table 8.1.

S.No.	Plant name	Weight of dried petals W_0 (gm)	Weight of empty petri plate W_1 (gm)	Weight of petri plate with plant extract W_2 (gm)	Percentage yield (%)
1.	*Rosa indica*	30 gm	47.800 gm	49.825 gm	6.75

Table No. 8.1: The percentage yield of crude methanolic petals extracts of *R. indica*, extraction done by soaking dried plant material in methanol and extract separation using distilling apparatus.

8.5 Total Phenolics Estimation of Petals Extracts

The total phenolic content was estimated spectrophotometrically using the Folin-Ciocalteu reagent at 765 nm. A calibration curve was drawn using Gallic Acid which was used as a standard. The level of gallic acid in the methanolic petals extract of *R. indica* was measured. 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, gallic acid is taken as standard in the study. 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 *R. indica* methanolic petals extract was estimated to be 10.65 GAE/g. The standard calibration curve is shown in Figure 8.2.

8.6 Tannins Estimation of Petals Extract

The total tannins content was also estimated spectrophotometrically at 765 nm using Folin-Denis Reagent using tannic acid as standard. The total tannins 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 methanolic petals extract for *R. indica* was estimated to be 2.88 TAE/g. The standard calibration curve is shown in Figure 8.3.

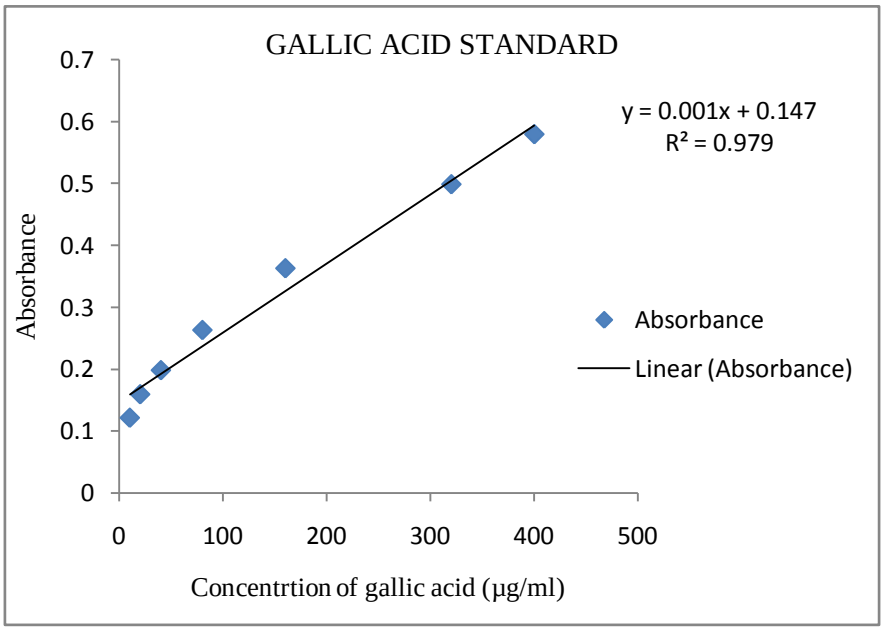

Figure No. 8.2: Calibration curve for gallic acid for determining the phenolic content.

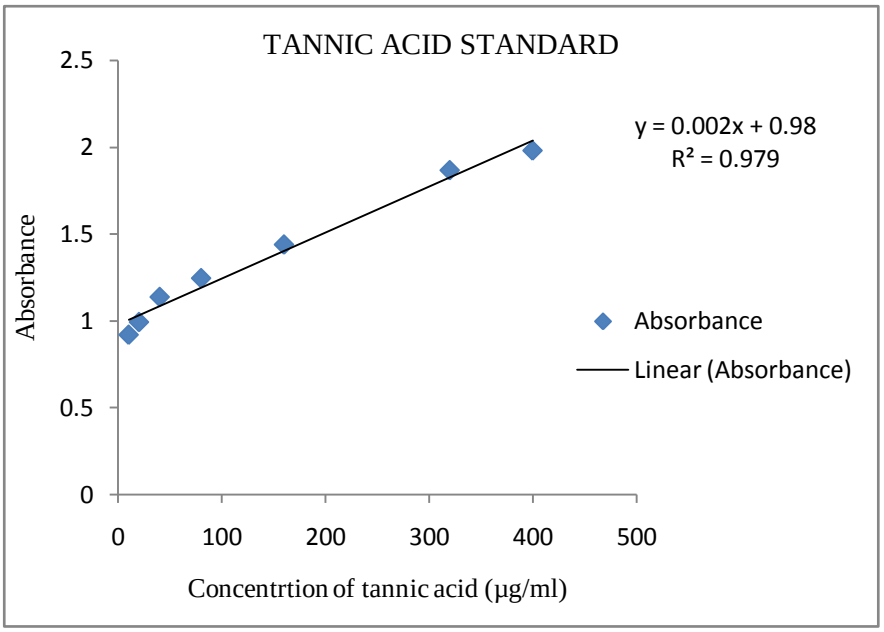

Figure No. 8.3: Calibration curve for tannic acid for determining the tannin content.

8.7 Phyto-chemcial Analysis of *Rosa indica*

The methanolic extract of petals of *Rosa indica* was tested qualitatively to analyze the presence of secondary metabolites. The secondary metabolites present in plants are alkaloids, anthraquinones, flavonoids, phlobatanins, glycosides, saponins, steroids, tannins and terpenoids. The methanolic extract of petals of *Rosa indica* showed positive results when analyzed using different phyto-chemical tests indicating the presence of the phyto-constituents.

The presence of alkaloid was analysed by using methanolic extract of petals with Wagner's method. The presence of reddish brown coloured precipitate indicates the presence of alkaloids. When the methanolic extract of *R. indica* was evaluated using this assay the presence of reddish brown coloured precipitate confirms the presence of alkaloids.

The Borntrager's test was performed for the analysis of anthraquinones in the methanolic petals extract. The formation of rose pink colour in plant extract confirmed the presence of anthraquinones. The methanolic petals extract when tested using this assay confirmed the appearance of pink colour indicating the presence of anthraquinones.

The presence of flavonoids in the crude plant extract is determined quantitatively, the appearance of yellow colour is the positive indication for the presence of flavonoids in them. When the crude methanolic petals extract of *R. indica* was evaluated using this test the appearance of yellow colour indicates the presence of flavonoids.

The phlobatannins presence was evaluated qualitatively by adding 1% of aqueous HCl in boiled crude methanoilic extract of *R. indica* petals, the presence of red colour indicates a positive result. The crude methanolic extracts of *R. indica* petals showed the presence of red colour indicating the presence of phlobatannins.

The presence of glycosides in the *R. indica* methanolic petals extract evaluated using the Fehling's test. The brick red precipitate formation indicates the presence of glycosides. *R. indica* methanolic petals extract showed the presence of brick red precipitate thus confirming the presence of glycosides in crude extract.

Similarly, the presence of saponins in the plant extract evaluated using a frothing test. The

S.No.	Active principle	Phyto-chemical Analysis	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. 8.2: Phyto-chemical analysis of flower extract of *Rosa indica*

formation of froth confirmed the presence of saponins. The *R. indica* petals extract showed the appearance of froth indicating the presence of saponins in the crude extract.

The *R. indica* crude methanolic petals extract 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 showed the change in colour indicating the presence of steroids in the extract.

The crude petals extract was further tested for the presence of tannins by using ferric chloride test. The occurrence of blue black precipitate indicates the presence of tannins. The *R. indica* methanolic petals extract showed the formation of blue black precipitate thus confirming the presence of tannins.

Similarly, Salkowski test was also performed to evaluate the presence of terpenoids in *R. indica* crude methanolic petals extract. The formation of reddish brown colour indicates the presence of terpenoids. The petals extract confirmed the presence of terpenoids as the reddish brown colour appeared in it.

8.8 Anti-oxidant Activities of Petals Extract of *Rosa indica*

Anti-oxidant activity of the methanolic crude petals extract of *R. indica* was determined *in vitro* by super oxide scavenging activity by using different assays such as alkaline DMSO method, DPPH free radical scavenging activity, nitric oxide free radical

scavenging activity, H_2O_2 radical scavenging activity and by total anti-oxidant capacity method.

8.8.1 Scavenging of Superoxide Radical with the Alkaline DMSO (dimethyl sulfoxide) Method

The superoxide radical scavenging assay, were studied in crude methanolic petals extracts 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 extracts. The increase in percentage showed stronger inhibition and highest scavenging activity of the plant extract.

The percentage inhibition values of *R. indica* petal extract were found to range between 71.94 ± 0.039 and 3.11 ± 0.476 percent, at the concentration of 1000 and 1.95 µg/ml respectively, whereas the percentage inhibition values of standard (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 *R. indica* extracts along with standard (BHT) at different concentrations are shown in Table 8.3 and 8.4 and Figure 8.4.

The crude methanolic petal extract of *R. indica* showed the ability to scavenge the super oxide radical and thus inhibits formazan formation. In Table 8.3 it is shown that increase scavenging of superoxide radicals in dose dependent manner due to the scavenging ability of the *R. indica* methanolic crude extract. The IC_{50} value of *R. indica* petal extract was found to be 157.2 ± 2.667 µg/ml, whereas the IC_{50} value of BHT was found to be 792.49 ± 1.16 µg/ml.

8.8.2 Nitric Oxide Free Radical Scavenging Activity

The *R. indica* methanolic crude petal extracts were also evaluated using the nitric oxide free radical scavenging activity. The standard used for the study was butylated hydroxytoluene (BHT). The methanolic extract of *Rosa indica* petals extract showed significant scavenging activity, and the percentage inhibition ranges between 52.74 ± 0.205 and 0.265 ± 0.265 percent, at the concentration of 1000 µg/ml and 1.95 µg/ml respectively whereas the percentage inhibition values of standard (BHT) were found to be 56.44 ± 0.113 and 1.90 ± 0.380 percent, at the

concentration of 1000 µg/ml and 1.95 µg/ml respectively. The nitric oxide radical scavenging activity values of the methanolic extracts along with standard at different concentrations are given in Table 8.5 and 8.6 and Figure 8.5. The IC_{50} value of *R. indica* petal extract was found to be 883.23 ± 4.40 µg/ml, whereas the IC_{50} value of BHT was found to be 364.60 ± 3.51 µg/ml.

8.8.3 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, determined according to the method done by Ruch *et. al.,* (1989), whereas compared with BHT, a positive control. The *R. indica* extracts were capable of scavenging hydrogen peroxide in a concentration dependent manner.

The crude methanolic petal extracts exhibited 55.35 ± 0.210 and 0.851 ± 0.557 percent inhibition at the concentration of 1000 and 1.95 µg/ml respectively, by hydrogen peroxide Anti-oxidant method. On the other hand, using the same concentration butylated hydroxy toluene exhibited 77.03 ± 0.128 and 4.14 ± 0.128 percent inhibition by hydrogen peroxide scavenging activity. The percentage inhibition values of methanolic petals extracts of *R. indica* and standard (BHT) were shown in Table 8.7 and 8.8 and Figure 8.6. The IC_{50} value of *R. indica* petal extract was found to be 863.33 ± 2.24 µg/ml, whereas the IC_{50} value of was BHT 26.16 ± 0.351 µg/ml.

8.8.4 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 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 results are shown as percentage inhibition values of the extracts at different concentrations ranging from 1.95 to 1000 µg/ml. The crude methanolic extracts of *R. indica* petals gave percent inhibition of 71.82 ± 0.246 and 1.374 ± 0.004 when tested at the concentration of 1000 and 1.95 µg/ml respectively, of the plant extract which was comparative with standard (BHT), used as standard having percent inhibition of 73.03 ± 0.128 and 12.59 ± 0.128, at the same concentration of 1000 µg/ml and 1.95 µg/ml respectively. The DPPH radical scavenging activity values of the methanolic extracts along with standard (BHT) had shown in Table 8.9 and 8.10 and Figure 8.7. The high percentage inhibition indicates high scavenging activity of the plant extract. The IC_{50} value of *R. indica* was found to be 154.06 ± 3.592 µg/ml. whereas the IC_{50} value of BHT was found to be 43.40 ± 1.307 µg/ml.

8.8.5 Total Anti-oxidant Capacity by Phosphomolybdenum Method

The total anti-oxidant capacity of the crude methanolic petal extracts and BHT were also determined by using phosphomolybdenum method. The higher absorbance value indicates the greater anti-oxidant activity. The total anti-oxidant capacity of plant extracts were measured spectrophotometrically at 695 nm using phosphomolybdenum method, which is based on the reduction of Mo (IV) to Mo (V) by the sample analyte and the subsequent 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 percentage inhibition values of the *R. indica* crude methanolic petal extracts

were found to be 53.30 ± 0.929 and 0.41 ± 0.712 percent, 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 concentrations of 1000 µg/ml and 1.95 µg/ml respectively. The values of the methanolic petals extracts along with standard (BHT) of total anti-oxidant capacity by phosphomolybdenum method were shown in Table 8.11 and 8.12 and Figure 8.8. The high percentage inhibition indicates high scavenging activity of the plant extract. The IC_{50} value of *R. indica* petal extract was found to be 823.75± 3.06 µg/ml. whereas the IC_{50} value of BHT was found to be 124.25 ± 3.04 µg/ml.

8.9 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 were conducted such as DPPH, Alkaline DMSO, Nitric oxide scavenging assay, total anti-oxidant assay and hydrogen peroxide method. The results are given below in Table 8.13.

S.No.	Plant conc. (µg/ml)	*Rosa indica* (Absorbance)	Percent Inhibition (%)
1.	1000	0.406 ± 0.000	71.94 ± 0.039
2.	500	0.300 ± 0.000	62.08 ± 0.072
3.	250	0.254 ± 0.001	55.11 ± 0.176
4.	125	0.220 ± 0.000	48.33 ± 0.135
5.	62.5	0.201 ± 0.001	43.28 ± 0.282
6.	31.25	0.185 ± 0.000	38.48 ± 0.191
7.	15.625	0.161 ± 0.001	29.19 ± 0.439
8.	7.8125	0.135 ± 0.001	15.55 ± 0.625
9.	3.906	0.128 ± 0.000	11.39 ± 0.398
10.	1.95	0.117 ± 0.000	3.11 ± 0.476

Table No. 8.3: The scavenging effect of methanolic petals extract of *R. indica* by Alkaline DMSO method. 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)	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. 8.4: The scavenging effect of BHT by Alkaline DMSO method. 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)	*Rosa indica* (Absorbance)	Percent Inhibition (%)
1.	1000	0.178 ± 0.001	52.74 ± 0.205
2.	500	0.222 ± 0.000	40.97 ± 0.243
3.	250	0.245± 0.001	34.95 ± 0.283
4.	125	0.262 ± 0.001	30.44 ± 0.285
5.	62.5	0.299 ± 0.001	20.61 ± 0.291
6.	31.25	0.312 ± 0.002	17.16 ± 0.425
7.	15.625	0.322 ± 0.001	14.33 ± 0.266
8.	7.8125	0.345 ± 0.001	8.40 ± 0.393
9.	3.906	0.355 ± 0.001	5.66 ± 0.403
10.	1.95	0.375 ± 0.000	0.265 ± 0.265

Table No. 8.5: The nitric oxide radical scavenging activity of methanollic petals extract of *R. indica*. 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	1.9 ± 0.380

Table No. 8.6: The nitric oxide radical scavenging activity of standard. The different concentrations of standards 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)	*Rosa indica* (Absorbance)	Percent Inhibition (%)
1.	1000	0.122 ± 0.000	55.35 ± 0.210
2.	500	0.176 ± 0.001	35.76 ± 0.364
3.	250	0.195 ± 0.001	28.83 ± 0.364
4.	125	0.201 ± 0.001	26.64 ± 0.364
5.	62.5	0.210 ± 0.001	23.35 ± 0.364
6.	31.25	0.222 ± 0.001	18.97 ± 0.364
7.	15.625	0.234 ± 0.001	14.35 ± 0.557
8.	7.8125	0.244 ± 0.003	10.70 ± 1.114
9.	3.906	0.252 ± 0.002	7.78 ± 0.759
10.	1.95	0.271 ± 0.001	0.85 ± 0.557

Table No. 8.7: The hydrogen peroxide radical scavenging activity of methanollic petals extract of *R. indica*. 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. 8.8: The hydrogen peroxide radical scavenging activity of standard**.** The different concentrations of standard 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)	*Rosa indica* (Absorbance)	Percent Inhibition (%)
1.	1000	0.082 ± 0.001	71.82 ± 0.246
2.	500	0.108 ± 0.001	62.88 ± 0.422
3.	250	0.121 ± 0.001	58.41 ± 0.200
4.	125	0.153 ± 0.001	47.42 ± 0.460
5.	62.5	0.191 ± 0.001	34.36 ± 0.302
6.	31.25	0.211 ± 0.000	27.37 ± 0.433
7.	15.625	0.229 ± 0.000	21.19 ± 0.335
8.	7.8125	0.241± 0.001	17.18 ± 0.543
9.	3.906	0.261 ± 0.001	10.30 ± 0.035
10.	1.95	0.287 ± 0.001	1.37 ± 0.004

Table No. 8.9: The DPPH radical scavenging activity of methanollic petals extract of *R. indica*.The different concentrations of extracts used from 1000 to 1.95 µg/ml. 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. 8.10: The DPPH radical scavenging activity standard. The different concentrations of extracts used from 1000 to 1.95 µg/ml. The standard used was butylated hydroxytoluene. Values are expressed as mean ± SD (n=3).

S.No.	Plant conc. (µg/ml)	*Rosa indica* (Absorbance)	Percent Inhibition (%)
1.	1000	0.037 ± 0.000	53.30 ± 0.929
2.	500	0.046 ± 0.001	42.96 ± 1.610
3.	250	0.050 ± 0.000	37.19 ± 0.267
4.	125	0.054 ± 0.000	32.23 ± 0.231
5.	62.5	0.057 ± 0.001	28.92 ± 0.635
6.	31.25	0.060 ± 0.000	25.20 ± 0.642
7.	15.625	0.064 ± 0.000	19.83 ± 0.120
8.	7.812	0.070 ± 0.000	12.80 ± 0.672
9.	3.906	0.075 ± 0.000	6.61 ± 0.691
10.	1.95	0.080 ± 0.000	0.41 ± 0.712

Table No. 8.11: Total Anti-oxidant Capacity of methanollic petals extract of *R. indica*. The different concentrations of extracts used from 1000 to 1.95 µg/ml. 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. 8.12: Total Anti-oxidant Capacity of standard. The different concentrations of extracts used from 1000 to 1.95 µg/ml. The standard used was butylated hydroxytoluene. Values are expressed as mean ± SD (n=3).

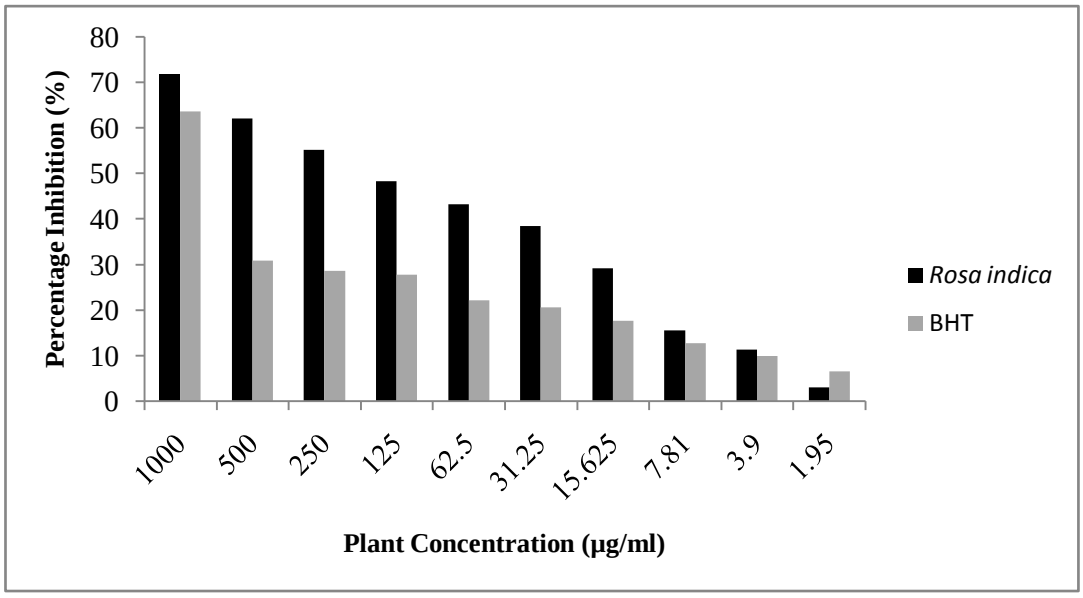

Figure No. 8.4: Graphical representation of percent inhibition of methanolic extract of petals of *Rosa indica* and Butylated hydroxy toluene (BHT) as a standard by using Alkaline DMSO method.

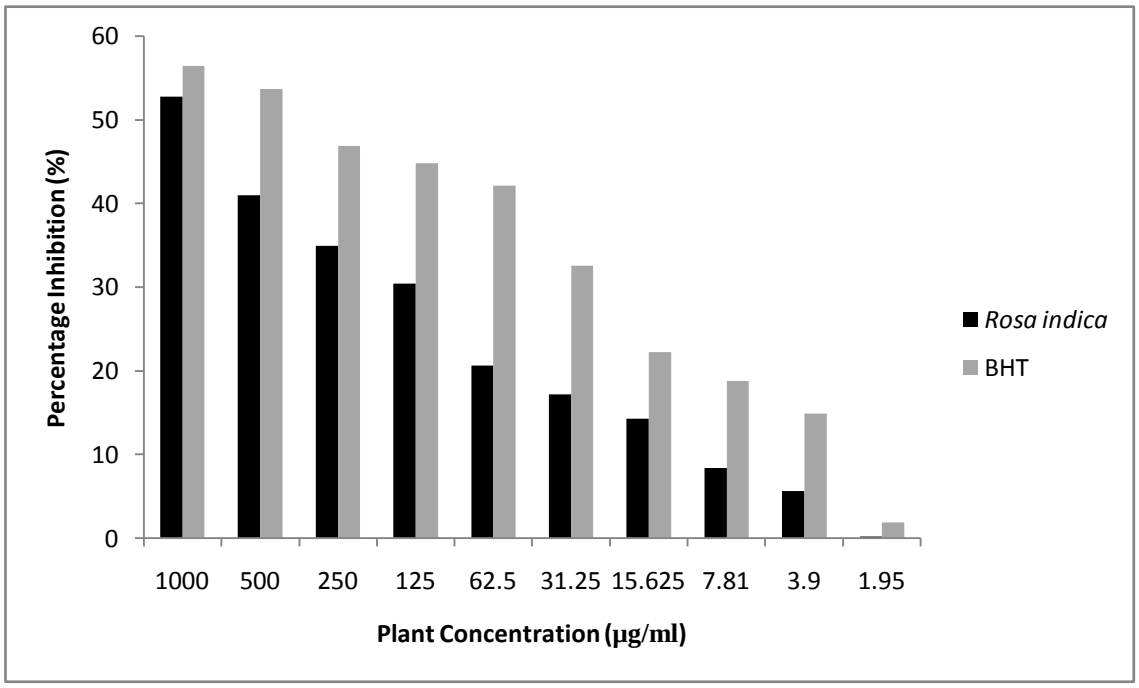

Figure No. 8.5: Graphical representation of percent inhibition of methanolic extract of petals of *Rosa indica* and Butylated hydroxy toluene (BHT) as standard by using nitric oxide radical scavenging activity**.**

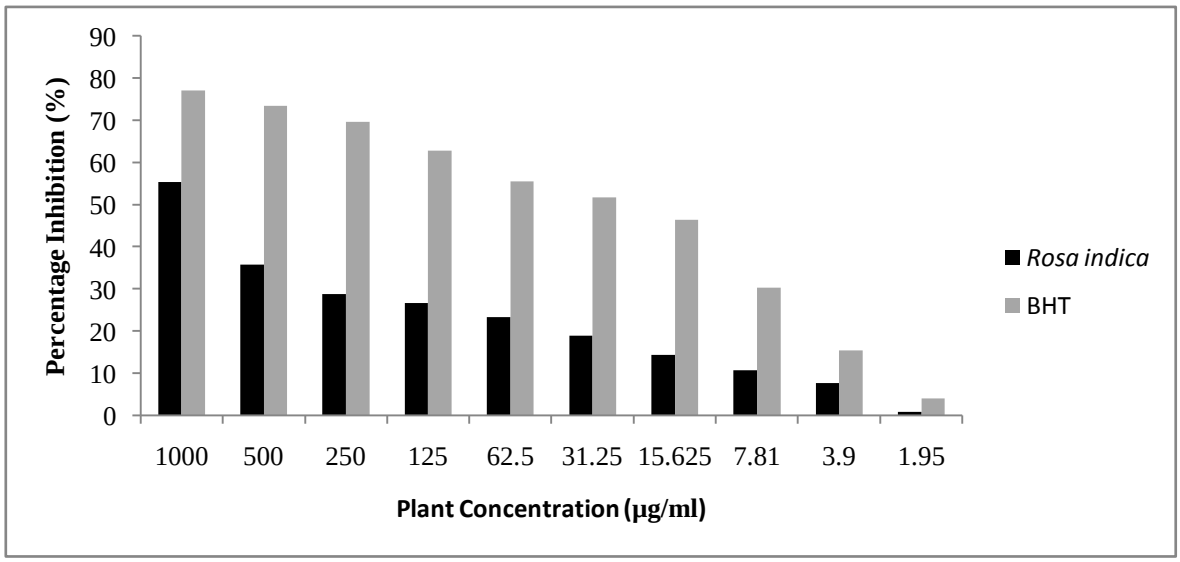

Figure No. 8.6: Graphical representation of percent inhibition of methanolic extract of petals of *Rosa indica* and Butylated hydroxy toluene (BHT) as standard by using hydrogen peroxide scavenging method**.**

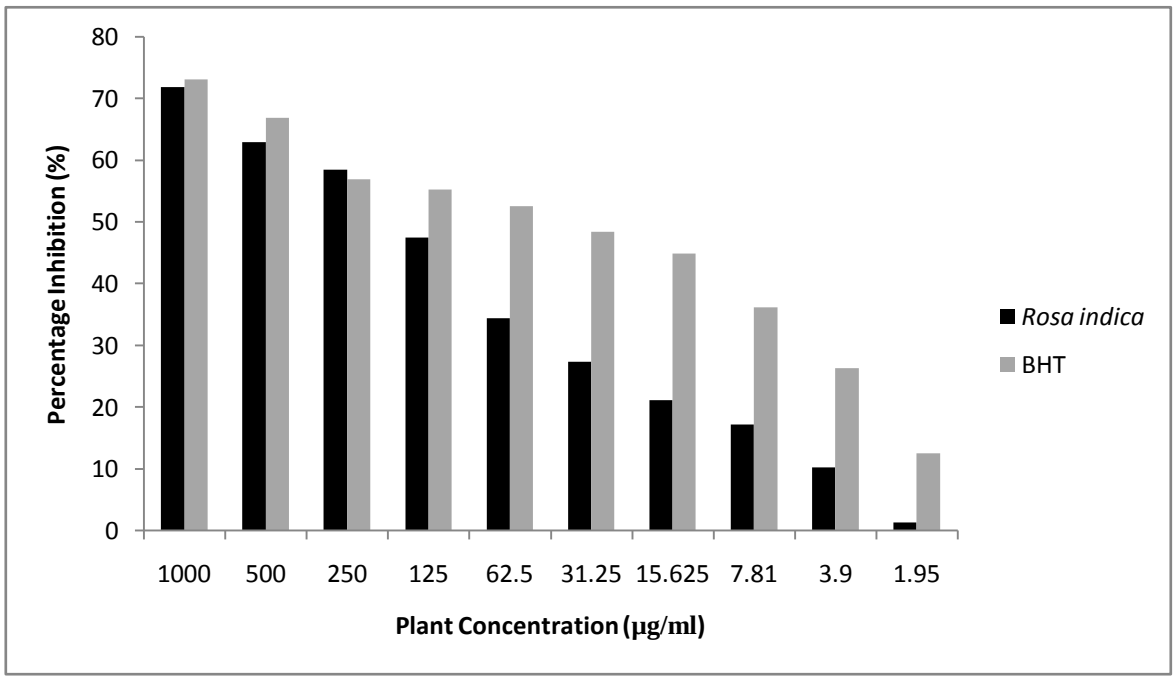

Figure No. 8.7: Graphical representation of percent inhibition of methanolic extract of petals of *Rosa indica* and Butylated hydroxy toluene (BHT) as standard by using DPPH radical scavenging activity**.**

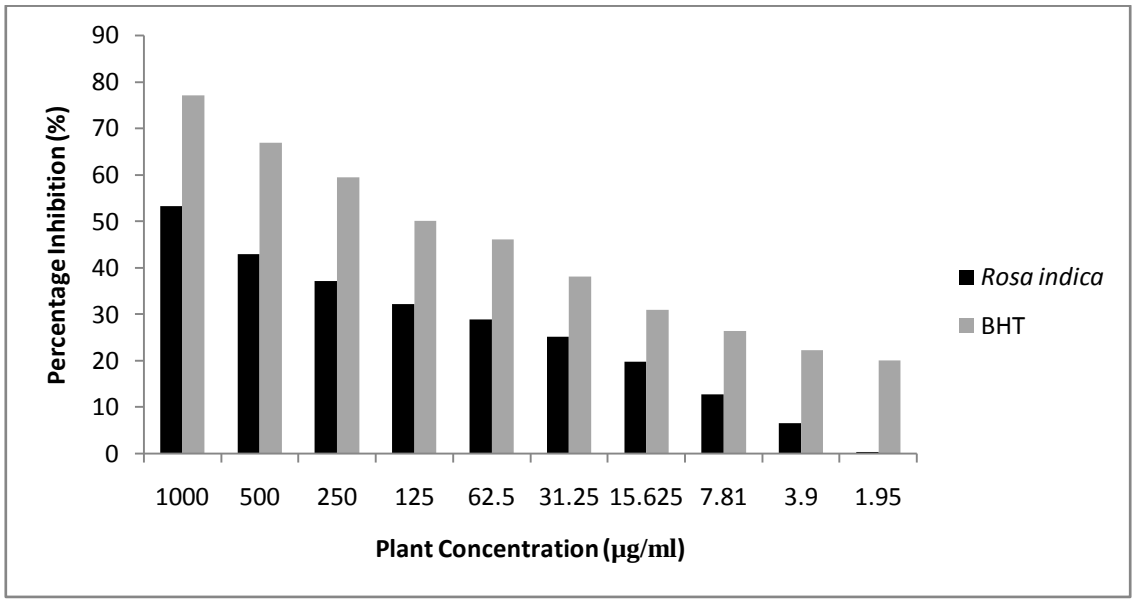

Figure No. 8.8: Graphical representation of percent inhibition of methanolic extract of petals of *Rosa indica* and Butylated hydroxy toluene (BHT) as standard by using total anti-oxidant capacity.

S.No.	Test Performed	IC_{50} value for Petals Extract	Butylated Hydroxytoluene
1.	Alkaline DMSO Method	157.2 ± 2.667	792.49 ± 1.16
2.	DPPH Method	154.06 ± 3.592	43.40 ± 1.307
3.	H_2O_2 Method	863.33 ± 2.24	26.166 ± 0.351
4.	Nitric Oxide Method	883.23 ± 4.40	364.60 ± 3.510
5.	Total Anti-oxidant Capacity Method	823.75± 3.06	124.25 ± 3.04

Table No.8.13: IC_{50} value of different anti-oxidant activity of methanolic petals extracts of *R. indica* and standard. 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).

8.10 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 methanolic extract of petals of *Rosa indica* such as alkaloids, phenols and flavonoids. The separation depends on the relative affinity of compounds towards stationary and mobile phase. The 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 *R. indica* methanolic extract of petals. 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. The yellow to purplish pink colour spots or rockets were observed. The retention factor (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.

The thin layer chromatogram was prepared by using 2 µg/ml of methanolic extract of petals of *Rosa indica* and standard compounds on silica gel plates. The R_f values of the extract and standard were calculated, observed 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.

$$R_f \text{ value} = \frac{\text{Distance travelled by component}}{\text{Distance travelled by solvent}}$$

The R_f value of methanolic extract of petals of *Rosa indica* was observed to be 0.73. The R_f value of gallic acid and tannic acid are 0.82 and 0.97. Thus, it indicates the presence of phenolics present in the *R. indica* petals extract. Apart from these, other bands were also seen indicating the presence of other compounds as well. The results are shown in Figure 8.9.

8.11 GC-MS Analysis of Methanolic Extract of Petals of *Rosa indica*

The crude methanolic petals extract of *R. indica* was characterized using GC-MS analysis to evaluate the compounds present in it. The results of GC-MS analysis showed that at least 39 compounds are present in methanolic extract of *R. indica*. These compounds which were identified through mass spectrometry are attached with GC. The mass spectra of these compounds were matched with those found in the NIST05 and WILEY 8 spectral database. The fragmentation of major compound was found in 1, 3, 4, 5-Tetrahydroxy-cyclohexanecarboxyl 28.24% (retention time: 12.599 min), 2-Furancarboxaldehyde, 5-(hydroxymethyl)- 21.05% (retention time: 8.438 min) and 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl- (retention time: 7.059 min) 18.76%. The active principles along with their retention time, area, area percent and compound name in the methanolic extract of *R. indica* are given in Table no. 8.14. The chromatogram of GC-MS is given in Figure 8.10.

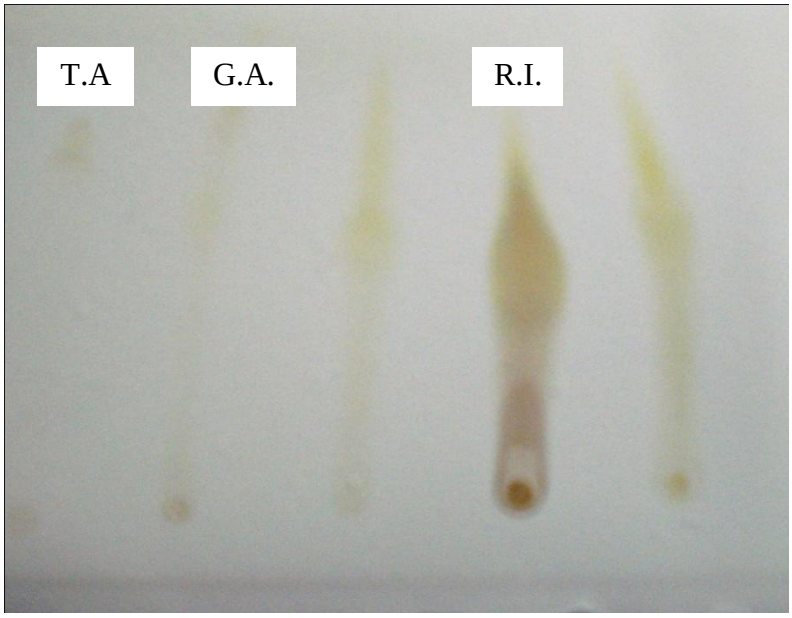

Figure No. 8.9: Thin Layered Chromatographic analysis of *Rosa indica*

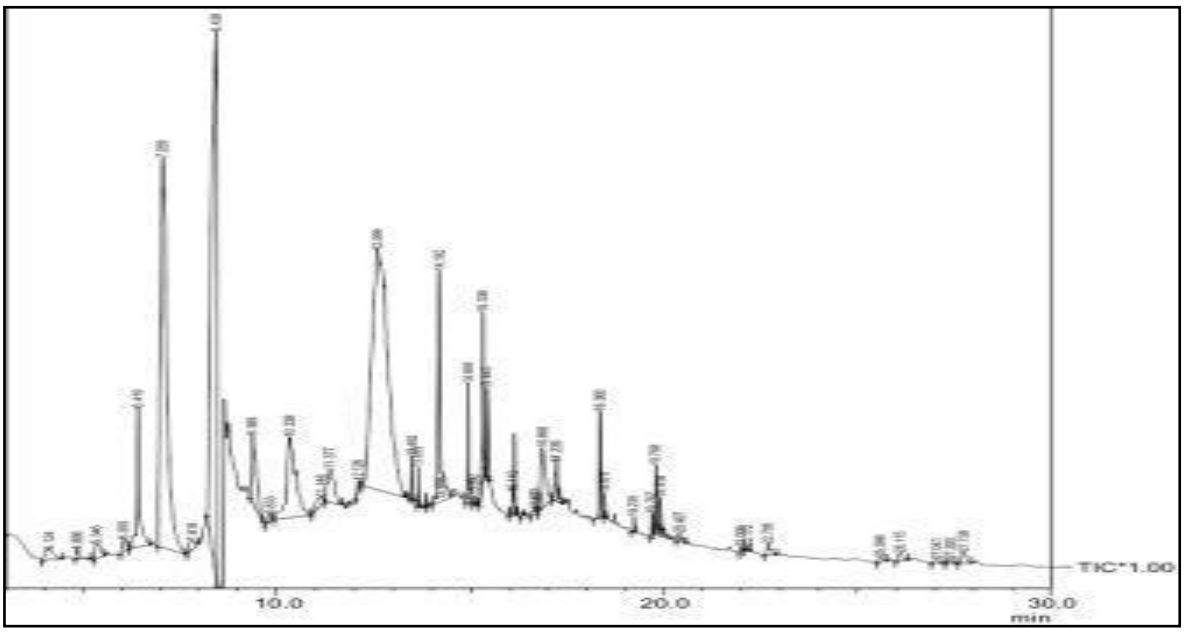

Figure No. 8.10: Chromatogram of *Rosa indica* methanolic extract of petals**.**

Peak	R. Time	Area	Area%	Name
1	4.124	10403131	0.69	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
2	4.856	3396321	0.23	1-Hexanol, 2-Ethyl-
3	5.346	9002758	0.60	Pentanoic Acid, 4-Oxo-
4	6.055	5777297	0.38	Ethanone, 1-(2-Furanyl)-2-Hydroxy-
5	6.419	41656414	2.77	Phenylethyl Alcohol
6	7.059	281993687	18.76	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
7	8.438	316431783	21.05	2-Furancarboxaldehyde, 5-(hydroxymethyl)-
8	9.385	36336963	2.42	cis-dimethyl morpholine
9	9.833	3337709	0.22	Propanal, 3-Ethoxy-
10	10.338	80945902	5.39	1,2,3-Benzenetriol
11	11.148	8786140	0.58	d-Glycero-d-ido-heptose
12	11.377	23811428	1.58	.beta.-D-Glucopyranose, 1,6-anhydro-
13	12.128	2422453	0.16	3-Buten-2-ol, 4-(2,6,6-Trimethyl-1-Cyclohexen-1-yl)-
14	12.599	424503770	28.24	1,3,4,5-Tetrahydroxy-Cyclohexanecarboxylic Acid
15	13.492	4286777	0.29	9-Eicosene, (E)-
16	13.653	3754958	0.25	Nonadecane
17	13.858	1375663	0.09	Hexadecanoic Acid, Methyl Ester
18	14.192	54157832	3.60	n-Hexadecanoic acid
19	14.959	15345648	1.02	Heneicosane
20	15.336	32006189	2.13	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
21	15.443	10328708	0.69	Octadecanoic acid
22	16.355	619996	0.04	trans-2-Dodecen-1-ol, heptafluorobutyrate
23	16.868	34385321	2.29	13-[(1-Phenylethylimino)methyl]tricyclo [8.2.2.24,7] hexadeca-1(13),4,6,10(14),
24	17.239	11756101	0.78	Tetratriacontane
25	18.360	16207270	1.08	Oxalic acid, hexadecyl 2-phenylethyl ester
26	18.475	2720315	0.18	6-Octen-1-ol, 3,7-dimethyl-, propanoate
27	19.235	2062653	0.14	Squalene
28	19.707	2921172	0.19	9-(1-phenylethoxy)-9-borabicyclo[3.3.1]nonane
29	19.798	9424393	0.63	Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6,6-dimethyl-
30	19.918	4898126	0.33	Dodecanoic acid, 2-phenylethyl ester

31	20.407	3835142	0.26	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-,
32	22.009	902482	0.06	1,4-Methanophthalazine, 1,4,4a,5,6,7,8,8a-octahydro-1,4,9,9-tetramethyl-,
33	22.172	782973	0.05	Oxalic acid, decyl 2-phenylethyl ester
34	22.706	4749428	0.32	dl-.alpha.-Tocopherol
35	25.598	2162853	0.14	Oxalic acid, hexadecyl 2-phenylethyl ester
36	26.115	4134946	0.28	gamma.-Sitosterol
37	27.041	1969432	0.13	A'-Neogammacer-22(29)-ene
38	27.393	1703780	0.11	Lupeol
39	27.738	6297987	0.42	6S-2,3,8,8-Tetramethyltricyclo[5.2.2.0(1,6)]undec-2-ene

Table No. 8.14: The peak results of *Rosa indica* methanolic petal extract.

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