CHAPTER – 9

SOLANUM NIGRUM

9.1 Introduction

Solanum nigrum is a species in the *Solanum genus* (Khattak *et. al.*, 2012). In India it is commonly known as black nightshade in english and makoi in hindi. The other vernacular names of *S. nigrum* include black or black berry nightshade in Australia, annual nightshade, common nightshade or garden nightshade in Europe, schwarzer nachtschatten in German, morelle noire in French, solanonero, solatro in Italian, paslen cernyj in Russian. In New Zealand, South Africa and West Indies the plant is called Black Nightshade (Edmonds and Chweya, 1997).

9.2 Description of the Plant

The *S. nigum* plant is sub-glabrous to villous annual which can grow up to 120 cm high, covered with simple multi-cellular hairs with glandular or non-glandular heads. The stem of the plant is erect but sometimes decumbent. The measurement of the leaves are 2.5-7.0 cm long and 2.0 to 4.5 (6.0) cm broad, entire margin entire. The leaves are broad, ovata, ovata-lanceolate, ovata-rhombic to lanceolate, margins entire to sinuate-dentate, somewhat heart shaped, with wavy or large-toothed edges (Edmonds and Chweya, 1997 and Khattak *et. al.*, 2012).

The calyces of the plant measures from 1.2 mm to 2.5 mm in length, it is slightly accrescent, adhering or deflexed to base of ripped berry, sepals usually ovata. The corollas are white in colour with translucent basal star, and are stellate. The radius of the corolla is from 5 to 7 mm, it is about 3 times longer than the calyx. The anthers of the plant are yellow in colour and are 1.5 to 2.5 mm in length. The pollens of the plant are small and the diameter measures from 29 to 34 μm. The styles are 2.8 to 3.5 mm in length, not exerted beyond the anthers. The berries are usually broadly ovoid, dull purple to blackish or yellowish-green, 6-10 mm broad, remaining on plants or falling from calyces when ripened. The inflorescence is simple and lax, it is often extended cymes, and it has five flowers, the number of flowers increases up to ten. The colour of the petals is greenish to

Figure 9.1: *Solanum nigrum*

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Solanales
Family : Solanaceae
Genus : *Solanum*
Species : *nigrum*

whitish, peduncles measuring from 14 up to 28 mm. It is usually erecto-patent, the length of the pedicels is short and after certain age the petals reoccur and surround anthers which are bright yellow in colour (Edmonds and Chweya, 1997 and Solanum, 2010).

9.3 Geographical Distribution

The *S. nigum* is an annual plant having 21 species of this genus is present in the flora. Some are crop weeds or weeds of rural habitats. The most widespread weed species found in Europe and Yugoslavia. It even belongs to South-Eurasian (Mediterranean) floral element and today is a cosmopolitan species (Quattrocchi, 2000). It grows in different types of habitats, as weed in crops and in rural habitats, along the roads, fences and usually in neglected places. Mostly semi cultivated in most countries and largely utilized as a vegetable and fruit source through harvesting from plants growing spontaneously as weeds in cultivated fields, or in weedy plant communities, under trees, along fences and roads, in shaded areas, near buildings and on waste land. Some communities, semi cultivates the vegetable in home gardens or on fertile land portions near homesteads (Herrera-Arellano *et. al.,* 2004).

9.4 Ethno-medical Properties and Uses

The *S. nigrum* has been used as anti-septic, anti-inflammatory, expectorant, cardio-tonic, digestive, diuretic, laxative, diaphoretic, sedative, swelling, cough, asthma, in curing cardio-pathy, leprosy, haemorrhoids, nephropathy, ophthalmopathy, dropsy and general debility. The plant has protective effect on the liver and hepato-protective activity in cases of toxicity induced by drugs and chemicals. It is also effective in the treatment of cirrhosis of the liver. Fresh juice of this herb is used for curing fever and alleviating pain (Solanum, 2006).

All the parts of the plant are very important as all the parts like seeds, leaves, flowers, possess medicinal value. The juice prepared from the leaves of *S. nigrum* used for skin problems and tumours. The seeds are used in home cosmetic, as it helps in removing freckles by simply rubbing the seeds on the cheeks. Decoctions made of stalk, leaves, roots are known to be effective on the wounds and also on cancerous sores. An infusion prepared by the plant is also proved to be very helpful in infants suffering from abdominal problems. Freshly prepared extract of the plant is effective in the treatment of cirrhosis of

the liver and also serves as a solution to opium poisoning. The poultice prepared by the leaves are used by the local practitioner for the treatment of rheumatic and gouty joints, skin diseases and also given in the treatment of tuberculosis. The leaves are also given in dropsy, nausea and nervous disorders. The juice made of berries of the plant used as anti-diarrhoea. It shows significant results in heart disease. The berries have tonic, diuretic and cathartic properties. The seeds are useful in giddiness and dypsea. The roots are also equally important as they also used in the treatment of various diseases like osteopathy, ophthalmopathy, rhinopathy and hepatitis. It has been reported earlier that aerial parts of *S. nigrum* is believed to have shown the anti-ulcer action through acid and peptic suppression in aspirin induced ulcerogenesis in rats (Solanum, 2010).

9.5 Phyto-constituents from *Solanum nigrum*

Many studies have shown that the plant of *S. nigrum* contains phyto-chemical constituents. A study done by Hussain *et. al.*, (1992) conducted phyto-chemical analysis and proved that the plant possess alkaloids, flavonoids, tannins, saponins, glycosides, proteins, carbohydrates, coumarins and phytosterols. The chemical characterization of osmotin like protein was also conducted from the plant. High concentration of solasodine was estimated from small unripe fruits of *S. nigrum* but a clear decrease was shown in the concentration of solasodine as well as in the absolute amount per fruit with fruit maturation (Kirtikar and Basu, 1935 and Nadkarni, 1976).

Studies conducted on the berries of *S. nigrum* showed the presence of 4 steroidal alkaloid glycosides, Solamargine, Solasonine, α solanigrine and β-solanigrine. The berries of *S. nigrum* also contain a saturated steroidal genin, which has been further identified as tigogenin by the method of mixed melting point and IR spectroscopy. In a study the methanolic extract prepared by the stems and roots of *S. nigrum* confirms the presence of one spirosestanol glycoside and two furostanol glycosides (Ravi *et. al.*, 2009a).

Six new steroidal saponins, solanigrosides C-H and one known saponin, degalactotigonin, were isolated from the whole plant of *S. nigrum*. Some researchers isolated two new steroidal saponins, named Nigrumnins I and II, together with two known saponins were obtained from the whole plant of *S. nigrum*. A phyto-chemical analysis conducted on the whole plant of *S. nigrum* has showed the presence of two new disaccharides. Their structures were determined as ethyl b-D-thevetopyranosyl- (1-4) -b-D-oleandropyranoside

and ethyl b-D-thevetopyranosyl- (1-4) -a-Doleandropyranoside, respectively, by chemical and spectroscopic methods. *S. nigrum* seeds are rich in lipid content. They also have high quantity of proteins and minerals (Mg being prominent) and oil extracted from the plant of *S. nigrum* is an important source of linoleic acid (Ravi *et. al.,* 2009b).

9.6 Pharmacology of *Solanum nigrum*

The extracts of *S. nigrum* had shown significant suppressive quality against the oxidant mediated DNA-sugar damage and the plant also showed potent activity of cyto-protection against gentamicin-induced toxicity on Vero cells. It has also prove that the plant have anti-neoplastic activity against Sarcoma 180 in mice. Studies done on the plant had also revealed that the plant extract possess an inhibitory effect on 12-Otetradecanoylphorbol 13-acetate (TPA) induced tumor promotion in HCT-116 cells. The ethanolic extract of dried fruit *S. nigrum* showed hepatoprotective activity against CCl_4-induced liver damage in mice. It has been reported that water extract of *S. nigrum* contains various anti-oxidants, like catechin, caffeic acid, gallic acid, PCA, epicatechin, rutin and narigenin. (Khattak *et. al.,* 2012).

The ethanolic extract of the fruit of *S. nigrum* was studied for its neuro-pharmacological properties on experimental animals. On intra-peritoneal injection, the extract significantly prolonged pentobarbital induced sleeping time, produced alteration in the general behaviour pattern and also reduced exploratory behaviour pattern, suppressed the aggressive behaviour, affected locomotors activity and reduced spontaneous motility. The observations suggest that the fruit of *S. nigrum* possesses potential CNS-Dependent action (Singh *et. al.,* 2001).

A study conducted on the water extract of *S. nigrum* showed protective effects against liver damage on chronic hepato-toxicity in rats induced with carbon tetrachloride (CCl_4). The extracts contributed to its modulation on detoxification enzymes and its anti-oxidant and free radical scavenger effects. Oral administration of *S. nigrum* significantly reduces thio-acetamide-induced hepatic fibrosis in mice (Heo and Lim, 2004).

One of the study showed that the plant possesses glycoprotein which had shown strong

scavenging effect against reactive oxygen radicals and growth inhibition effects against JA221 and XL1-Blue. The glycoprotein had shown cytotoxic effects against MCF-7 and HT-29 cells, even at low concentrations also (Jainu and Devi, 2006) The other study done by number of scientists reported glycoprotein had a strong scavenging activity against ROS, lipid peroxyl radicals and hypo-lipidemic activity by increasing the detoxicant enzymes activity through the inhibition of hepatic HMG-CoA reductase in mice (Abbas *et. al.,* 1998, Ahmad *et. al.*, 2007 and Akhtar and Muhammad, 1989). Glycoprotein had the tendency that it can modulate the TPA-induced DNA-binding activities of transcription factors and NO production (Al-Qirim *et. al.,* 2008 and Jamil *et. al.*, 2007).

Akbar and Munir (1989) reported that 50% ethanolic extract of the whole plant of *S. nigrum* showed cytoprotection against gentamicin-induced toxicity on Vero cells by the trypan blue exclusion and mitochondrial dehydrogenase activity (MTT) assay. The sample extract also exhibited significant scavenging activity by hydroxyl radical scavenging assay. The plant also possess anti-secretory activity which is known to be mainly associated with the inhibition of H+K+ATPase and suppression of gastrin release, while the ulcer protective and ulcer healing activities showed by the *S. nigrum* plant is supposed to be initially related to an anti-secretary effect of *S. nigrum* (Jainu and Devi, 2004). The anti-oxidant potential of leaves extract of *S. nigrum* was evaluated on the method of restraint induced oxidative stress (Seithe and Anderson, 1982 and Veseliaia, 1974). The study showed that the post treatment of crude extract was found more effective in restoring restraint stress induced oxidative changes in rat plasma than pre-treatment. Glycoprotein had shown potent anti-oxidant potential by several methods like DPPH, superoxide radical and hydroxyl radical assay (Kumar *et. al.,* 2001 and Takhtajan, 1997).

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

The initial weight of 30 gm of the dried berries, leaves and flowers of *Solanum nigrum* were taken in 100 ml of methanol. The percentage yield obtained in the berries of *S. nigrum* was found to be 6 percent, while the percentage yield obtained in the flower of *S. nigrum* was found to be 3.6 percent whereas the percentage yield obtained in the leaves of *S. nigrum* was found to be 5.23 percent. The percentage yield of extracts of berries flowers and leaves of *S. nigrum* in methanol is given below in Table 9.1.

S.No.	*Solanum nigrum*	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.	Berries	30 gm	48.700 gm	50.500 gm	6%
2.	Flowers	30 gm	45.100 gm	46.180 gm	3.6%
3.	Leaves	30 gm	46.700 gm	48.270 gm	5.23%

Table No. 9.1: Percentage yield of methanolic plant extracts, extraction done by soaking dried plant material in methanol and extract separation using distilling apparatus.

9.8 Total Phenolics Estimation of Berries, Leaves and Flowers Extract

The total phenolic content was estimated spectrophotometrically using the Folin-Ciocalteu Reagent at 765 nm. A calibration curve was drawn using Gallic Acid as a standard. The level of Gallic acid in the methanolic berries, leaves and flowers extract of *S. nigrum* 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. 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 *S. nigrum* berries, leaves and flowers extracts was found to be 10.79 GAE/g, 9.39 GAE/g and 6.54 GAE/g respectively. The standard calibration curve is shown in Figure 9.2.

9.9 Tannins Estimation of Berries, Leaves and Flowers Extract

The total tannins content was also estimated spectrophotometrically at 765 nm using Folin-Denis reagent here tannic acid was used 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:

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

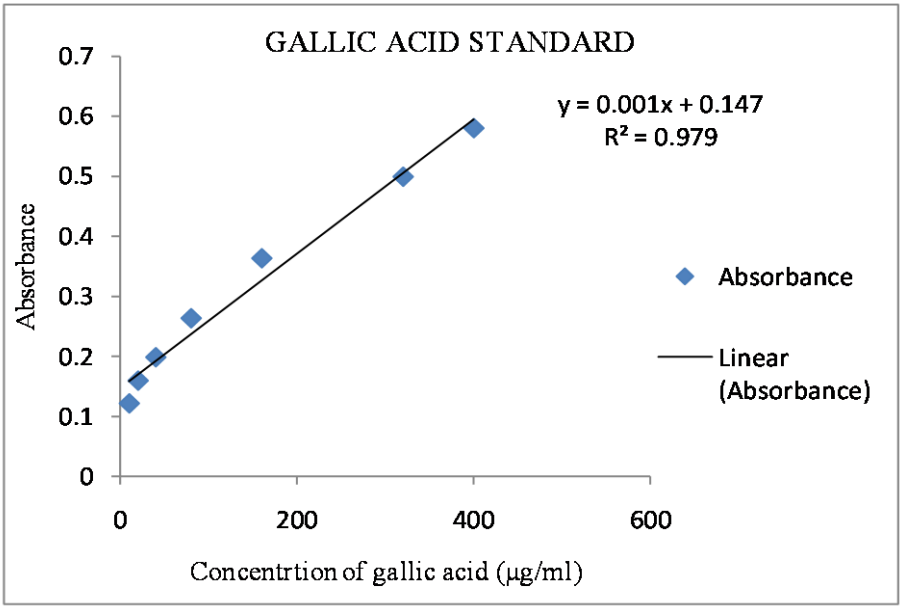

Figure No. 9.2: Calibration curve for gallic acid for determining the phenolic content.

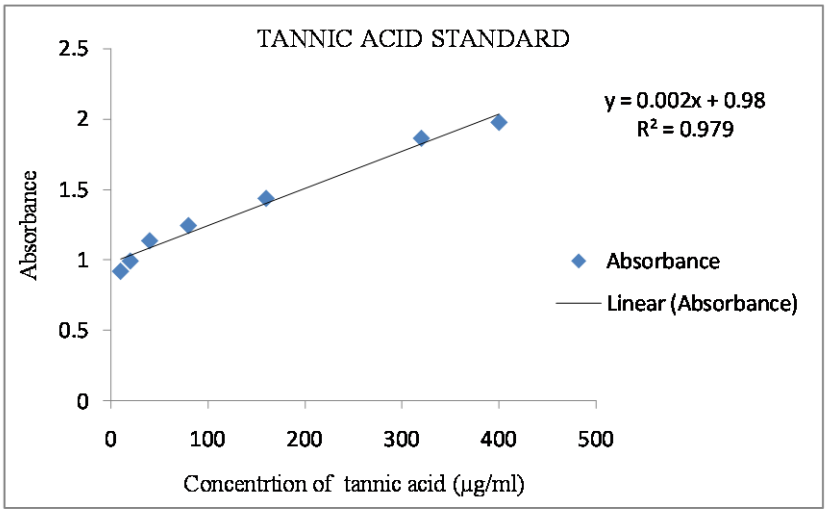

Figure No. 9.3: Calibration curve for tannic acid for determining the tannin content.

The experiment was replicated thrice and average data recorded for quality assurance. The TAE for methanolic seed extract of *L. royleana* was estimated to be 0.18 TAE/g. The standard calibration curve is shown in Figure 9.3.

The TAE for methanolic berries, leaves and flowers extracts of *S. nigrum* was found to be 3.64 TAE/g, 3.19 TAE/g and 2.42 TAE/g respectively.

9.10 Phyto-chemical Analysis of Berries, Leaves and Flowers Extract of *S. nigrum*

The phyto-chemical analysis involves qualitative analysis of herbal plants. The preliminary qualitative tests have been attempted in crude extracts of *Solanum nigrum* berries, leaves and flowers extracts, to find out the presence or absence of certain bio-active compounds.

The secondary metabolites present in the crude methanolic extract of berries are alkaloids, flavonoids, phlobatanins, glycosides, saponins, steroids, tannins and terpenoids. While the secondary metabolites present in crude methanolic extract of leaves were found to be alkaloids, flavonoids, phlobatanins, glycosides, saponins, steroids and tannins. Whereas, the secondary metabolites present in the crude methanolic extract of flowers are alkaloids, phlobatanins, glycosides, saponins and tannins. The *S. nigrum* crude methanolic extract of berries, leaves and flowers showed positive result to the different phyto-chemical tests indicating the presence of a number of phyto-constituents. The results of qualitative phyto-chemical analysis are given in Table 9.2.

The presence of alkaloid in methanolic extract of berries, leaves and flowers were analysed by using Wagner's method. The presence of reddish brown coloured precipitate indicates the presence of alkaloids. When the crude methanolic berries, leaves and flowers extract of *S. nigrum* were evaluated using this assay the presence of reddish brown coloured precipitate was found which confirms the presence of alkaloids in all the three extracts of *S. nigrum*.

The Borntrager's test was performed for the analysis of anthraquinones in the methanolic extract of berries, leaves and flowers. The formation of rose pink colour in plant extract confirmed the presence of anthraquinones. The methanolic berries, leaves and flowers extract when tested using this assay and all the three extracts of *S. nigrum* did not give pink colour indicating the absence of anthraquinones in all the extracts.

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 methanolic extract of berries and leaves of *S. nigrum* were evaluated for the presence of flavonoids then the appearance of yellow colour indicates its presence in both methanolic berries as well as in the leaves extracts. Whereas, the methanolic flower extract of *S. nigrum* did not show the appearance of yellow colour indicating the absence of flavonoids.

The phlobatannins presence was evaluated qualitatively by adding 1% of aqueous HCl in boiled crude methanolic extract of *S. nigrum* berries, leaves and flowers. The presence of red colour indicates a positive result. The crude methanolic extracts of *S. nigrum* berries, leaves and flowers showed the presence of red colour indicating the presence of phlobatannins.

The presence of glycosides in the *S. nigrum* methanolic extract of berries, leaves and flowers was evaluated using the Fehling's test. The brick red precipitate formation indicates the presence of glycosides in the plant extract. The *S. nigrum* leaves, berries as well as flowers extract showed the presence of brick red precipitate thereby, confirming the presence of glycosides in all three crude extracts.

Similarly, the presence of saponins in *S. nigrum* berries, leaves and flowers extract were evaluated using frothing test. The formation of froth confirms the presence of saponins. The *S. nigrum* leaves and flowers extracts as well as berries extracts showed the appearance of froth indicating the presence of saponins in all three extracts.

The *S. nigrum* crude methanolic berries, leaves and flowers 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 extracts of leaves and flowers showed the change of colour from violet to blue thus confirming the presence of steroids in both the extracts, while the crude methanolic berries extract did not showed the change in colour indicating the absence of steroids.

The crude leaves, flowers and berries extracts 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 *S. nigrum* methanolic extract of berries, leaves and flowers 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 *S. nigrum* crude methanolic extract of leaves, berries and flower. The formation of reddish brown colour indicates the presence of terpenoid. The berries extract confirmed the presence of terpenoids whereas is it found to be absent in the leaves as well as in flowers extracts.

S.No.	Active principle	Tests for Phyto-constituents	Flower Extract Result	Leaves Extract Result	Berries 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. 9.2: Results of phyto-chemical analysis of *Solanum nigrum*

9.11 Anti-oxidant Activities of Berries, Leaves and Flowers Extract of *Solanum nigrum*

The anti-oxidant activity of methanolic extract of berries, leaves and flower of *S. nigrum* was determined *in vitro* by using a number of assays such as 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 by total anti-oxidant capacity method.

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

The superoxide radical scavenging assay, were studied in crude methanolic berries, leaves and flowers extract of *S. nigrum* 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 plant extract.

The percentage inhibition values of methanolic berries extract *S. nigrum* were found to be 74.36 ± 0.033 and 5.26 ± 0.453 percent, while the percentage inhibition values of methanolic leaves extract of *S. nigrum* were found to be 71.82 ± 0.144 and 5.78 ± 0.778 percent, whereas the percentage inhibition values of methanolic flower extract *S. nigrum* were found to be 70.86 ± 0.042 and 5.52 ± 0.453 percent, at the concentration of 1000 µg/ml and 1.95 µg/ml respectively. One the other hand, the percentage inhibition values of standard (BHT), which was taken as standard, were found to be 63.52 ± 0.020 and 6.63 ± 0.229 percent, at the concentration of 1000 µg/ml and 1.95 µg/ml respectively. The percentage inhibition values of *S. nigrum* berries, leaves and flowers extracts and standard (BHT) at different concentrations are shown in Table 9.3, 9.4, 9.5 and 9.6 and Figure 9.4.

The crude methanolic extract of *S .nigrum* scavenges super oxide radical and thus inhibits formazan formation. In Table 9.3, 9.4, 9.5 it is illustrated that increase in scavenging of superoxide radicals in dose dependent manner due to the scavenging ability of the *S .nigrum* methanolic extract. The IC_{50} value of *S. nigrum* berries extracts was found to be 223.8 ± 3.21 µg/ml, while the IC_{50} value of *S. nigrum* leaves extracts was found to be 171.46 ± 1.05 µg/ml, whereas the IC_{50} value of *S. nigrum* flowers extract was found to be 153.9 ± 2.78 µg/ml and the IC_{50} value of BHT was 792.49 ± 1.16 µg/ml.

9.11.2 Nitric Oxide Free Radical Scavenging Activity

The methanolic berries, leaves and flowers extracts were evaluated using the nitric

oxide free radical scavenging activity. The standard used for the study was butylated hydroxy toluene (BHT). The *S. nigrum* methanolic extracts showed significant scavenging activity.

The percentage inhibition values of *S. nigrum* berries extracts were found to be 75.86 ± 0.265 and 1.59 ± 0.265 percent, while the percentage inhibition values of *S. nigrum* leaves extracts were found to be 72.06 ± 0.405 and 0.53 ± 0.265 percent, whereas the percentage inhibition values of *S. nigrum* flowers extracts were found to be 70.55 ± 0.265 and 1.59 ± 0.265 percent, at the concentration of 1000 µg/ml and 1.95 µg/ml respectively. One the other hand, 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 percentage inhibition values of *S. nigrum* berries, leaves and flowers extracts along with standard (BHT) at different concentrations shown in Table 9.7, 9.8, 9.9 and 9.10. and Figure 9.5. The IC_{50} value of *S. nigrum* berries extracts was found to be 125.83 ± 4.06 µg/ml, while the IC_{50} value of *S. nigrum* leaves extracts was found to be 461.46 ± 2.54 µg/ml, whereas the IC_{50} value of *S. nigrum* flowers extract was found to be 196.56 ± 2.43 µg/ml and the IC_{50} value of BHT was 364.60 ± 3.51 µg/ml.

9.11.3 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), where they are compared with that of BHT. The extracts were capable of scavenging hydrogen peroxide in a concentration dependent manner.

The methanolic berries extracts exhibited 45.19 ± 0.308 and 1.42 ± 0.005 percent

inhibition, while methanolic leaves extracts exhibited 42.46 ± 0.345 and 1.42 ± 0.201 percent inhibition whereas the methanolic flower extracts exhibited 40.21 ± 0.496 and 1.06 ± 0.614 percent inhibition at the concentration of 1000 µg/ml 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 hydrogen peroxide radical scavenging activity values of the methanolic extracts of *S. nigrum* berries, leaves and flowers along with standard (BHT) at different concentrations were shown in Table 9.11, 9.12, 9.13 and 9.14 and Figure 9.6.

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

The DPPH radical scavenging 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 percentage inhibition values of *S. nigrum* berries extracts were found to be 73.28 ± 0.250 and 1.71 ± 0.005 percent, while the percentage inhibition values of *S. nigrum* leaves extracts were found to be 91.75 ± 0.371 and 0.91 ± 0.196 percent, whereas the percentage inhibition values of *S. nigrum* flowers extracts were found to be 81.09 ± 0.315 and 3.43 ± 0.586 percent, at the concentration of 1000 µg/ml and 1.95 µg/ml respectively.On the other hand, the percentage inhibition values of standard (BHT) were found to be 73.03 ± 0.128 and 12.59 ± 0.128 percent respectively, at the concentration of 1000 µg/ml and 1.95 µg/ml.

The DPPH radical scavenging activity values of the methanolic extracts of *S. nigrum* berries, leaves and flowers and standard (BHT) have shown in Table 9.15, 9.16, 9.17 and 9.18 and Figure 9.7. The percentage inhibition indicates scavenging activity of the plant extract. The IC_{50} value of methanolic berries extract of *S. nigrum* was found to be 190.7 ± 2.35 µg/ml, while the IC_{50} value of methanolic leaves extracts of *S. nigrum* was found to be 110.7 ± 1.609 µg/ml and

the IC_{50} value of methanolic flowers extracts of *S. nigrum* was found to be 155.133 ± 2.402 µg/ml whereas the IC_{50} value of BHT was found to be 43.40 ± 1.307 µg/ml.

9.11.5 Total Anti-oxidant Capacity by Phosphomolybdenum Method

Total anti-oxidant capacity of the plant extracts and BHT were determined by the using the method of Phospho-molybdenum. The total anti-oxidant capacity of plant extracts were measured spectrophotometrically at 695 nm. 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 attributed to their chemical composition and phenolic acid content.

The percentage inhibition values of methanolic berries extracts were found to be 48.51 ± 0.641 and 0.00 ± 0.000 percent, while the percentage inhibition values of methanolic leaves extracts were found to be 45.73 ± 0.106 and 0.213 ± 0.000 percent, The percentage inhibition values of methanolic flowers extracts were found to be 49.27 ± 0.176 and 0.23 ± 0.000 percent respectively. On the other hand, the percentage inhibition values of 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 berries, leaves and flowers extracts of *S. nigrum* and standard (BHT) of total anti-oxidant capacity by phospho-molybdenum method were shown in Table 9.19, 9.20, 9.21 and 9.22 and Figure 9.8.

9.12 IC_{50} value of Different Anti-oxidant Activity

The IC_{50} values of the methanolic berries, leaves and flowers extracts of *S. nigrum* were calculated based on the results of different anti-oxidant assay were conducted as DPPH, Alkaline DMSO, Nitric oxide scavenging assay, Total anti-oxidant assay and Hydrogen peroxide method. The results are given below in Table 9.23.

S.No.	Plant conc. (µg/ml)	*Solanum nigrum* (Berries) (Absorbance)	Percent Inhibition (%)
1	1000	0.444 ± 0.000	74.36 ± 0.033
2	500	0.291 ± 0.001	60.82 ± 0.134
3	250	0.233 ± 0.000	51.14 ± 0.120
4	125	0.211 ± 0.001	45.97 ± 0.256
5	62.5	0.200 ± 0.000	43.09 ± 0.163
6	31.25	0.187 ± 0.001	39.03 ± 0.326
7	15.625	0.161 ± 0.001	29.19 ± 0.439
8	7.8125	0.145 ± 0.001	21.37 ± 0.542
9	3.906	0.133 ± 0.001	14.28 ± 0.644
10	1.95	0.120 ± 0.000	5.26 ± 0.453

Table No. 9.3: The scavenging effect of methanolic berries extract of *S.nigrum* 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)	*Solanum nigrum* (Leaves) (Absorbance)	Percent Inhibition (%)
1	1000	0.404 ± 0.002	71.82 ± 0.144
2	500	0.310 ± 0.000	63.26 ± 0.068
3	250	0.265 ± 0.000	57.08 ± 0.093
4	125	0.210 ± 0.000	45.88 ± 0.148
5	62.5	0.198 ± 0.000	42.52 ± 0.167
6	31.25	0.182 ± 0.000	37.59 ± 0.197
7	15.625	0.163 ± 0.001	30.05 ± 0.429
8	7.8125	0.144 ± 0.000	21.19 ± 0.315
9	3.906	0.131 ± 0.001	12.97 ± 0.664
10	1.95	0.121 ± 0.001	5.78 ± 0.777

Table No. 9.4: The scavenging effect of methanolic leaves extract of *S.nigrum* 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)	*Solanum nigrum* (Flowers) (Absorbance)	Percent Inhibition (%)
1	1000	0.391 ± 0.000	70.86 ± 0.042
2	500	0.298 ± 0.000	61.83 ± 0.073
3	250	0.260 ± 0.001	56.20 ± 0.096
4	125	0.220 ± 0.000	48.26 ± 0.135
5	62.5	0.190 ± 0.001	40.20 ± 0.181
6	31.25	0.170 ± 0.000	33.20 ± 0.226
7	15.625	0.161 ± 0.001	29.19 ± 0.439
8	7.8125	0.151 ± 0.000	24.66 ± 0.286
9	3.906	0.133 ± 0.000	14.49 ± 0.369
10	1.95	0.120 ± 0.000	5.52 ± 0.453

Table No. 9.5: The scavenging effect of methanolic flowers extract of *S.nigrum* 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. 9.6: The scavenging effect of standard by Alkaline DMSO method. 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)	*Solanum nigrum* (Berries) (Absorbance)	Percent Inhibition (%)
1.	1000	0.091 ± 0.001	75.86 ± 0.265
2.	500	0.123 ± 0.001	67.37 ± 0.265
3.	250	0.153 ± 0.001	59.41 ± 0.265
4.	125	0.188 ± 0.001	50.13 ± 0.265
5.	62.5	0.233 ± 0.001	38.19 ± 0.265
6.	31.25	0.266 ± 0.001	29.44 ± 0.265
7.	15.625	0.311 ± 0.000	17.41 ± 0.153
8.	7.8125	0.335 ± 0.001	11.14 ± 0.265
9.	3.906	0.360 ± 0.001	4.33 ± 0.306
10.	1.95	0.370 ± 0.000	1.76 ± 0.153

Table No. 9.7: The nitric oxide radical scavenging activity of methanollic berries extract of *S. nigrum*. 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)	*Solanum nigrum* (Leaves) (Absorbance)	Percent Inhibition (%)
1.	1000	0.105 ± 0.001	72.06 ± 0.405
2.	500	0.180 ± 0.000	52.16 ± 0.153
3.	250	0.232 ± 0.000	38.37 ± 0.153
4.	125	0.266 ± 0.001	29.44 ± 0.265
5.	62.5	0.291 ± 0.001	22.81 ± 0.265
6.	31.25	0.311 ± 0.000	17.41 ± 0.153
7.	15.625	0.334 ± 0.002	11.31 ± 0.552
8.	7.8125	0.351 ± 0.001	6.80 ± 0.405
9.	3.906	0.361 ± 0.001	4.24 ± 0.265
10.	1.95	0.375 ± 0.001	0.53 ± 0.265

Table No. 9.8: The nitric oxide radical scavenging activity of methanollic leaves extract of *S. nigrum*. 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/gm)	*Solanum nigrum* (Flowers) (Absorbance)	Percent Inhibition (%)
1.	1000	0.111 ± 0.001	70.55 ± 0.265
2.	500	0.145 ± 0.001	61.53 ± 0.265
3.	250	0.177 ± 0.001	53.05 ± 0.265
4.	125	0.203 ± 0.002	46.15 ± 0.530
5.	62.5	0.234 ± 0.001	37.84 ± 0.306
6.	31.25	0.277 ± 0.001	26.52 ± 0.265
7.	15.625	0.300 ± 0.001	20.42 ± 0.265
8.	7.8125	0.323 ± 0.001	14.32 ± 0.265
9.	3.906	0.354 ± 0.000	6.01 ± 0.153
10.	1.95	0.371 ± 0.001	1.59 ± 0.265

Table No. 9.9: The nitric oxide radical scavenging activity of methanollic flowers extract of *S. nigrum*. 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. 9.10: The nitric oxide 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 nitric oxide inhibition. Values are expressed as mean ± SD (n=3).

S.No.	Plant conc. (µg/ml)	*Solanum nigrum* (Berries) (Absorbance)	Percent Inhibition (%)
1.	1000	0.154 ± 0.001	45.19 ± 0.308
2.	500	0.175 ± 0.002	37.72 ± 1.00
3.	250	0.192 ± 0.001	31.67 ± 0.315
4.	125	0. 206 ± 0.002	26.45 ± 0.995
5.	62.5	0.211 ± 0.001	24.91 ± 0.540
6.	31.25	0.221 ± 0.001	21.23 ± 0.758
7.	15.625	0.234 ± 0.001	16.48 ± 0.836
8.	7.812	0.254 ± 0.001	9.60 ± 0.585
9.	3.906	0.264 ± 0.002	5.81 ± 1.011
10.	1.95	0.277 ± 0.001	1.42 ± 0.005

Table No. 9.11: The hydrogen peroxide radical scavenging activity of methanollic berries extract of *S. nigrum***.** The different concentrations of extracts and standards 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)	*Solanum nigrum* (Leaves) (Absorbance)	Percent Inhibition (%)
1.	1000	0.161 ± 0.001	42.46 ± 0.345
2.	500	0.175 ± 0.002	37.72 ± 1.00
3.	250	0.191 ± 0.001	31.90 ± 0.308
4.	125	0.205 ± 0.001	27.04 ± 0.318
5.	62.5	0.210 ± 0.001	25.02 ± 0.489
6.	31.25	0.220 ± 0.000	21.58 ± 0.144
7.	15.625	0.234 ± 0.001	16.72 ± 0.564
8.	7.8125	0.254 ± 0.002	10.08 ± 0.751
9.	3.906	0.264 ± 0.001	6.04 ± 1.598
10.	1.95	0.277 ± 0.000	1.42 ± 0.201

Table No. 9.12: The hydrogen peroxide radical scavenging activity of methanollic leaves extract of *S. nigrum*. The different concentrations of extracts and standards 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)	*Solanum nigrum* (Flowers) (Absorbance)	Percent Inhibition (%)
1.	1000	0.168 ± 0.001	40.21 ± 0.496
2.	500	0.181 ± 0.001	35.34 ± 0.770
3.	250	0.191 ± 0.001	31.90 ± 0.308
4.	125	0. 204 ± 0.001	27.16 ± 0.422
5.	62.5	0.211 ± 0.001	24.79 ± 0.655
6.	31.25	0.220 ± 0.000	21.58 ± 0.144
7.	15.625	0.234 ± 0.001	16.72 ± 0.564
8.	7.8125	0.255 ± 0.002	9.25 ± 1.034
9.	3.906	0.264 ± 0.001	6.04 ± 0.598
10.	1.95	0.278 ± 0.001	1.06 ± 0.614

Table No. 9.13: The hydrogen peroxide radical scavenging activity of methanollic flowers extract of *S. nigrum*. The different concentrations of extracts and standards 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 ± 0.000	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. 9.14: The hydrogen peroxide 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 hydrogen peroxide inhibition. Values are expressed as mean ± SD (n=3).

S.No.	Plant conc. (µg/ml)	*Solanum nigrum* (Berries) (Absorbance)	Percent Inhibition (%)
1.	1000	0.078 ± 0.001	73.28 ± 0.250
2.	500	0.113 ± 0.001	61.18 ± 0.654
3.	250	0.121 ± 0.001	58.56 ± 0.298
4.	125	0.173 ± 0.001	40.63 ± 0.724
5.	62.5	0.209 ± 0.001	28.42 ± 0.587
6.	31.25	0.220 ± 0.000	24.54 ± 0.132
7.	15.625	0.234 ± 0.001	19.86 ± 0.534
8.	7.8125	0.254 ± 0.000	12.78 ± 0.358
9.	3.906	0.272 ± 0.001	6.84 ± 0.661
10.	1.95	0.286 ± 0.000	1.71 ± 0.005

Table No. 9.15: The DPPH radical scavenging activity of methanollic berries extract of *S.nigrum.* 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)	*Solanum nigrum* (Leaves) (Absorbance)	Percent Inhibition (%)
1.	1000	0.024 ± 0.001	91.75 ± 0.371
2.	500	0.086 ± 0.001	70.44 ± 0.242
3.	250	0.113 ± 0.001	60.93 ± 0.656
4.	125	0.135 ± 0.001	53.37 ± 0.369
5.	62.5	0.178 ± 0.001	38.83 ± 0.133
6.	31.25	0.213 ± 0.001	26.80 ± 0.308
7.	15.625	0.245 ± 0.001	15.80 ± 0.320
8.	7.8125	0.260± 0.000	10.53 ± 0.365
9.	3.906	0.276 ± 0.000	4.92 ± 0.382
10.	1.95	0.288 ± 0.000	0.91 ± 0.196

Table No. 9.16: The DPPH radical scavenging activity of methanollic leaves extract of *S.nigrum***.** 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)	*Solanum nigrum* (Flowers) (Absorbance)	Percent Inhibition (%)
1.	1000	0.055 ± 0.001	81.09 ± 0.315
2.	500	0.094 ± 0.001	67.69 ± 0.454
3.	250	0.114 ± 0.002	60.59 ± 0.730
4.	125	0.155 ± 0.001	46.73 ± 0.160
5.	62.5	0.202 ± 0.001	30.58 ± 0.582
6.	31.25	0.218 ± 0.001	25.08 ± 0.086
7.	15.625	0.238 ± 0.001	18.21 ± 0.540
8.	7.8125	0.250 ± 0.000	13.86 ± 0.477
9.	3.906	0.264 ± 0.001	9.27 ± 0.655
10.	1.95	0.288 ± 0.000	3.43 ± 0.586

Table No. 9.17: The DPPH radical scavenging activity of methanollic flowers extract of *S.nigrum* 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)	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. 9.18: 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)	*Solanum nigrum* (Berries) (Absorbance)	Percent Inhibition (%)
1.	1000	0.046 ± 0.000	48.51 ± 0.641
2.	500	0.050 ± 0.000	44.07 ± 0.641
3.	250	0.054 ± 0.000	39.25 ± 0.641
4.	125	0.060 ± 0.000	32.96 ± 0.641
5.	62.5	0.067 ± 0.000	25.18 ± 0.641
6.	31.25	0.070 ± 0.000	21.48 ± 0.641
7.	15.625	0.074 ± 0.000	17.40 ± 0.641
8.	7.812	0.081 ± 0.000	10.00 ± 0.000
9.	3.906	0.087 ± 0.000	2.59 ± 0.641
10.	1.95	0.09 ± 0.000	0.00 ± 0.000

Table No. 9.19: The total anti-oxidant activity by phosphomolybdenum method of methanollic berries extract of *S. nigrum*. 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)	*Solanum nigrum* (Leaves) (Absorbance)	Percent Inhibition (%)
1.	1000	0.254 ± 0.000	45.73 ± 0.106
2.	500	0.277 ± 0.000	40.75 ± 0.502
3.	250	0.300 ± 0.000	35.85 ± 0.176
4.	125	0.320 ± 0.000	31.57 ± 0.180
5.	62.5	0.355 ± 0.000	24.11 ± 0.188
6.	31.25	0.391 ± 0.000	16.57 ± 0.134
7.	15.625	0.411 ± 0.001	12.30 ± 0.131
8.	7.812	0.420 ± 0.001	10.24 ± 0.202
9.	3.906	0.450 ± 0.000	3.84 ± 0.209
10.	1.95	0.467 ± 0.000	0.213 ± 0.000

Table No. 9.20: The total anti-oxidant activity by phosphomolybdenum method of methanollic leaves extract of *S. nigrum*. 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)	*Solanum nigrum* (Flowers) (Absorbance)	Percent Inhibition (%)
1.	1000	0.220 ± 0.000	49.27 ± 0.176
2.	500	0.249 ± 0.000	42.59 ± 0.565
3.	250	0.255 ± 0.000	41.13 ± 0.115
4.	125	0.287 ± 0.000	33.84 ± 0.192
5.	62.5	0.301 ± 0.000	30.54 ± 0.118
6.	31.25	0.321 ± 0.000	25.94 ± 0.119
7.	15.625	0.350 ± 0.000	19.26 ± 0.240
8.	7.812	0.377 ± 0.000	13.04 ± 0.125
9.	3.906	0.400 ± 0.000	7.75 ± 0.255
10.	1.95	0.433 ± 0.000	0.23 ± 0.000

Table No. 9.21: The total anti-oxidant activity by phosphomolybdenum method of methanollic flowers extract of *S. nigrum*. 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)	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. 9.22: The total anti-oxidant activity by phosphomolybdenum method 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 inhibition values. Values are expressed as mean ± SD (n=3).

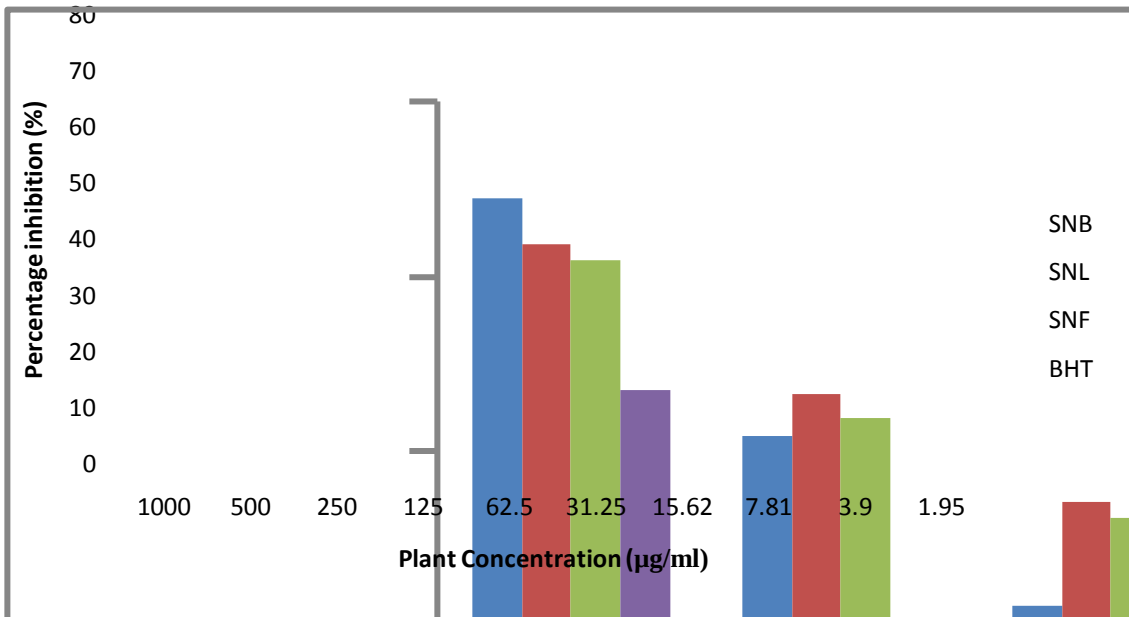

Figure No. 9.4: Graphical representation of percent inhibition of methanolic extract of berries (SNB), leaves (SNL) and flowers (SNF) of *Solanum nigrum* and butylated hydroxy toluene (BHT) as standard by using Alkaline DMSO method.

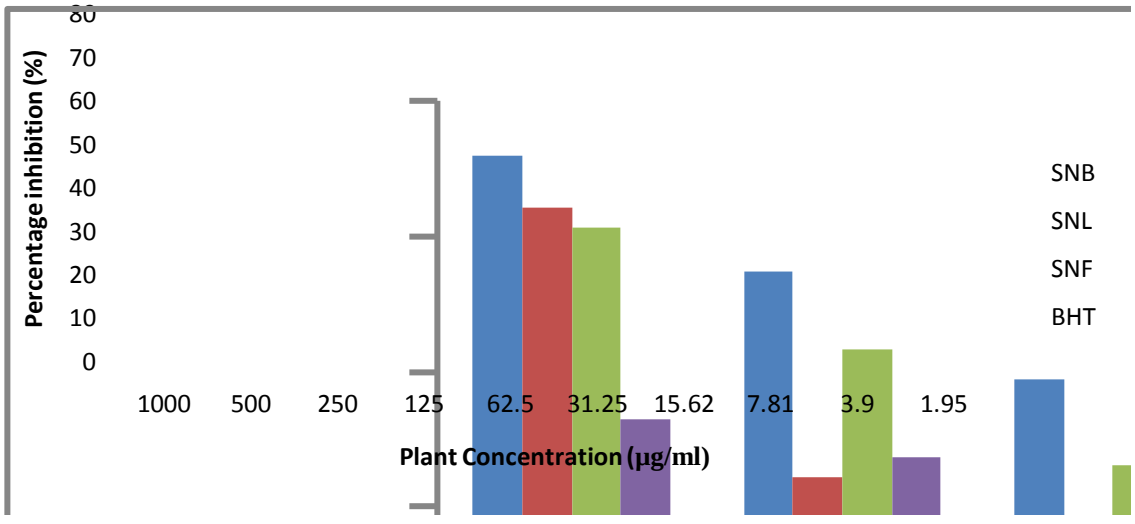

Figure No. 9.5: Graphical representation of percent inhibition of methanolic extract of berries (SNB), leaves (SNL) and flowers (SNF) of *Solanum nigrum* and Butylated hydroxy toluene (BHT) as standard by using nitric oxide radical scavenging activity**.**

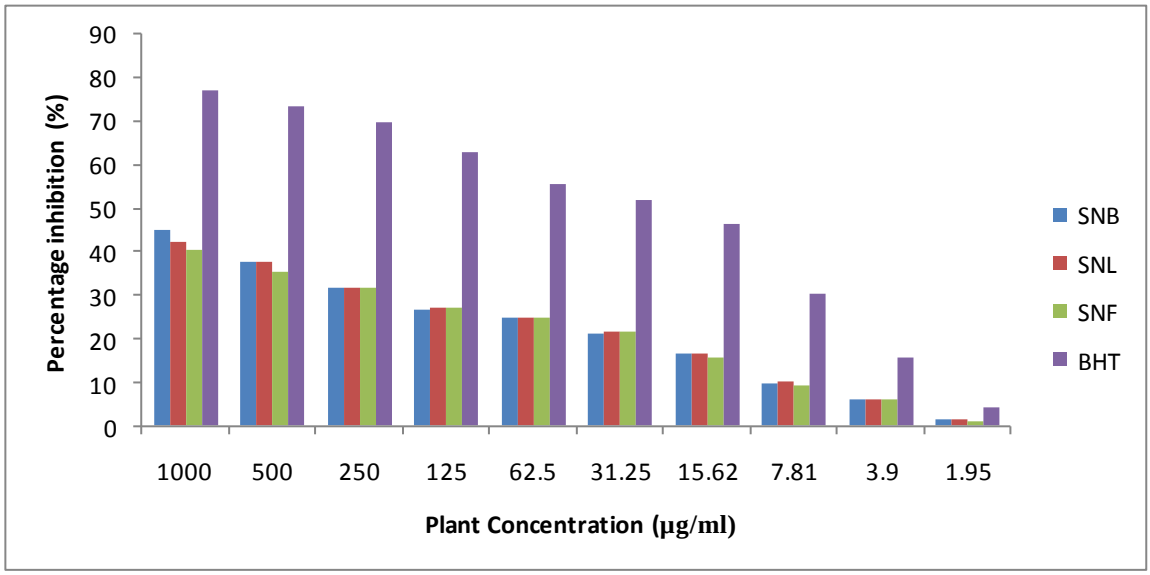

Figure No. 9.6: Graphical representation of percent inhibition of methanolic extract of berries (SNB), leaves (SNL) and flowers (SNF) of *Solanum nigrum* and Butylated hydroxy toluene (BHT) as standard by using hydrogen peroxide scavenging method**.**

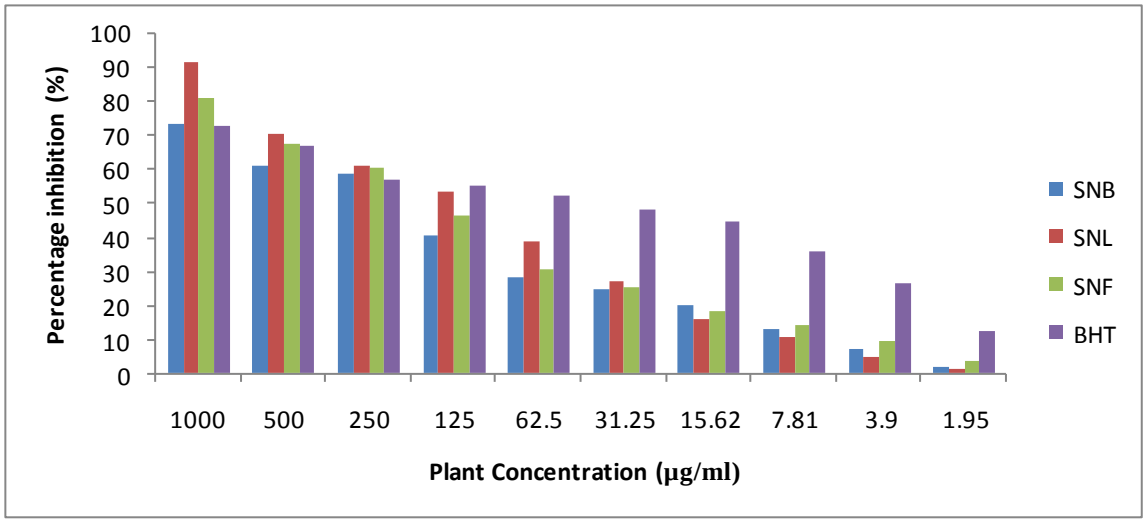

Figure No. 9.7: Graphical representation of percent inhibition of methanolic extract of berries (SNB), leaves (SNL) and flowers (SNF) of *Solanum nigrum* Butylated hydroxy toluene (BHT) as standard by using DPPH radical scavenging activity**.**

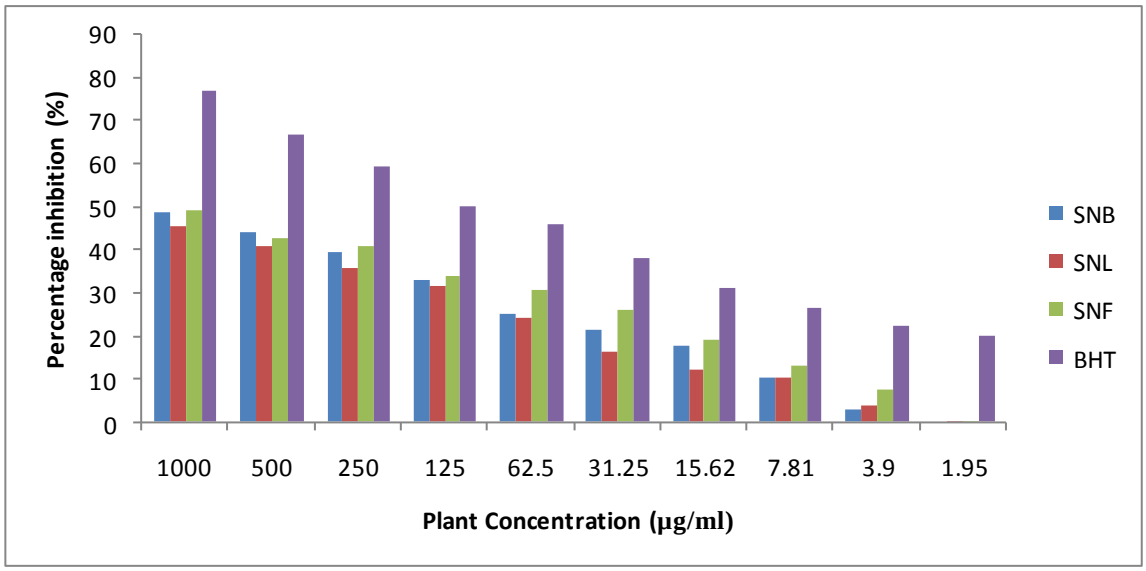

Figure No. 9.8: Graphical representation of percent inhibition of methanolic extract of berries (SNB), leaves (SNL) and flowers (SNF) of *Solanum nigrum* Butylated hydroxy toluene (BHT) as standard by using total anti-oxidant capacity.

S.No.	Test Performed	IC_{50} value for Berries Extract	IC_{50} value for Leaves Extract	IC_{50} value for Flower Extract	IC_{50} value for Butylated Hydroxytoluene
1.	Alkaline DMSO Method	223.8 ± 3.21*	171.46 ± 1.05*	153.9 ± 2.78	792.49 ± 1.16
2.	DPPH Method	190.7 ± 2.35*	110.7 ± 1.60*	155.13 ± 2.40*	43.40 ± 1.307
3.	H_2O_2 Method	>1000	>1000	>1000	26.166 ± 0.351
4.	Nitric Oxide Method	125.83 ± 4.06	461.46 ± 2.54*	196.56 ± 2.43*	364.60 ± 3.510
5.	Total Anti-oxidant Capacity Method	>1000	>1000	>1000	124.25 ± 3.04

Table No. 9.23: Comparative chart of 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)

9.12 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 *Solanum nigrum* methanolic berries and leaves extracts such as alkaloids, phenols, flavonoids, etc. 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 the *S. nigrum* methanolic berries 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. The yellow to purplish pink colour spots or rockets were observed. The retention factor (R_f) of the unknown coumpound is compared with R_f of the known compound (gallic acid and tannic acid). The R_f is the retention factor, indicating how far compound travelled on the silica gel plates.

The thin layer chromatogram was prepared by using 2 µg/ml of *S. nigrum* methanolic berries 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.

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

The R_f value of *S. nigrum* methanolic berries and leaves extract was observed to be 0.7 and 0.73 respectively. The R_f value of gallic acid and tannic acid were 0.88 and 0.91. Thus, it indicates there are phenolics present in the *S. nigrum* methanolic berries 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 9.9.

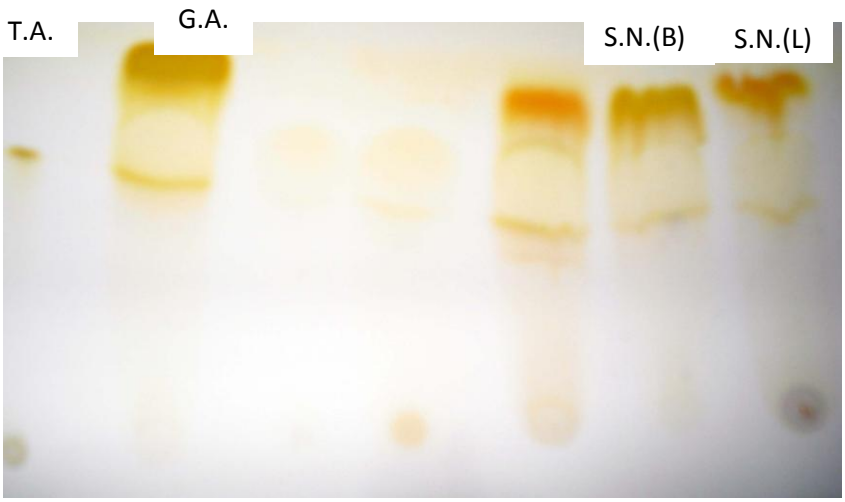

Figure No. 9.9: Thin Layered Chromatographic analysis of *Solanum nigrum*

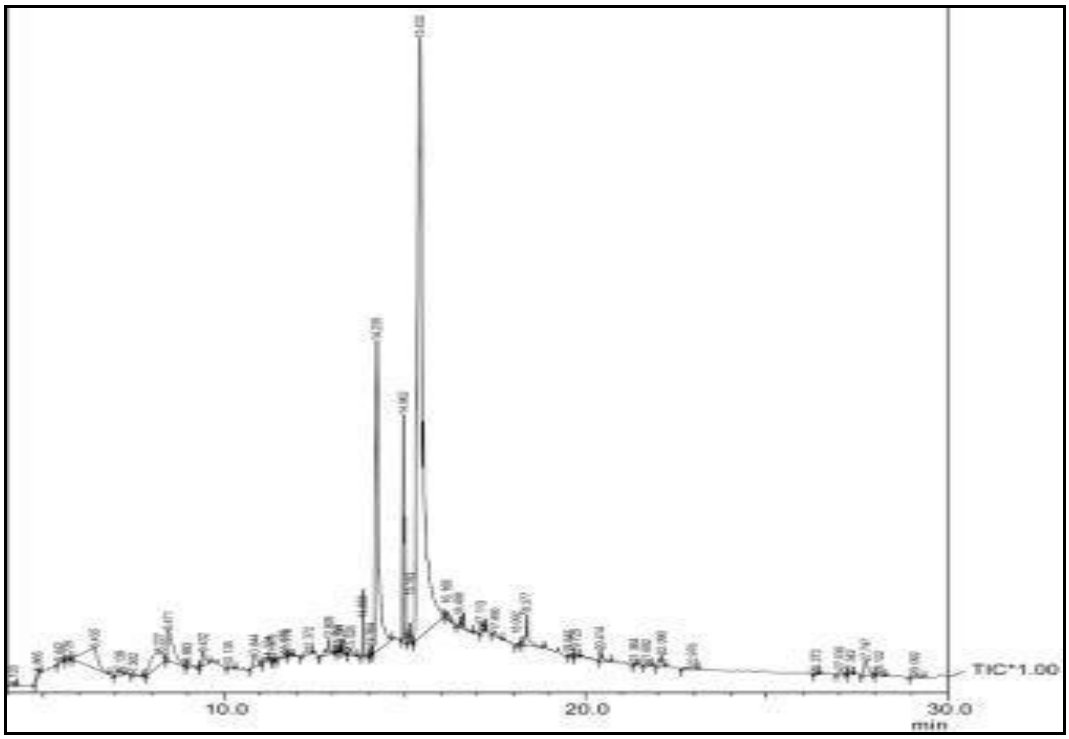

Figure No. 9.10: Chromatogram of *Solanum nigrum* methanolic extract of seeds.

9.14 GC-MS Analysis of *Solanum nigrum* Methanolic Extract

The crude methanolic berries extract of *S. nigrum* 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 *S. nigrum*. 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 9, 12-Octadecadienoic acid 54.31% (retention time: 15.432 min), n-Hexadecanoic acid 15.77% (retention time: 14.235 min) and 9, 12-Octadecadienoic acid (Z,Z)- (retention time: 14.962 min) 4.97%. The active principles along with their retention time, area, area percent and compound name in the methanolic extract of *S. nigrum* are given in Table 9.24. The chromatogram of GC-MS is given in Figure 9.10.

Peak	R. Time	Area	Area%	Name
1	4.133	1305873	0.11	1-Di(tert-butyl)silyloxyhexadecane
2	4.865	3560860	0.31	1-Hexanol, 2-Ethyl-
3	5.442	1878345	0.17	2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl-, (.+/-.)-
4	5.725	451463	0.04	Silane, 2-butenylmethoxymethylphenyl-
5	6.435	53225305	4.68	Glycerin
6	7.502	1455915	0.13	2,3-Anhydro-d-mannosan
7	8.227	31897866	2.80	2,5-Dimethyl-1-hepten-4-ol
8	8.471	38127349	3.35	2-butenoic acid, 2(OR 4)-Isooctyl-4,6(OR 2,6)-Dinitrophenyl 10 8.993 4241863 0.37
9	9.432	8970180	0.79	2-Decenoic Acid
10	10.135	2307242	0.20	1,5-Anhydro-l-rhamnitol
11	10.844	9567344	0.84	Lactone G
12	11.224	2045323	0.18	4-Hydroxy-3,5,5-trimethylcyclohex-2-enone
13	11.675	3306272	0.29	2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2-propenyl)-
14	11.776	2077685	0.18	13-Methylpentadec-14-ene-1,13-diol
15	12.909	8057601	0.71	Pentadec-7-ene, 7-bromomethyl-
16	13.158	1480071	0.13	6-(3-Methylbutyl)-2-Pyrazinylmethanol
17	13.231	2637654	0.23	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne
18	13.858	10544334	0.93	Hexadecanoic acid, methyl ester
19	14.064	3681800	0.32	4,6-Diamino-O-cresol
20	14.235	179411189	15.77	n-Hexadecanoic acid
21	14.962	56560995	4.97	9,12-Octadecadienoic acid (Z,Z)-, methyl ester

22	15.143	4642299	0.41	Octadecanoic acid, methyl ester
23	15.432	617977197	54.31	9,12-Octadecadienoic acid (Z,Z)-
24	17.113	4261082	0.37	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester
25	18.092	1021676	0.09	4a(2H)-Phenanthrenecarboxaldehyde, 1,3,4,9,10,10a-hexahydro-6-methoxy-1,1-
26	18.377	15687817	1.38	Tetratriacontane
27	19.545	1162443	0.10	Pentalene, Octahydro-1-(2-Octyldecyl)-
28	19.725	1972176	0.17	(1S,2E,4S,5R,7E,11E)-Cembra-2,7,11-trien-4,5-diol
29	20.414	5050949	0.44	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(
30	21.364	1017740	0.09	Cholesta-4,6-dien-3-ol, (3.beta.)-
31	21.692	1408237	0.12	7-Dehydrodiosgenin 3-acetate
32	22.090	6884326	0.61	Cholesta-4,6-dien-3-ol, (3.beta.)-
33	22.976	2818551	0.25	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.beta.,4.alpha.,5.alpha.)-
34	26.373	635119	0.06	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.beta.,4.alpha.,5.alpha.)-
35	27.039	3136533	0.28	4-(2,2-Dimethyl-6-Methylenecyclohexylidene)-3-Methyl-2
36	27.342	857485	0.08	1-(1,5-Dimethyl-4-Hexenyl)-3a,6,6,12a-Tetramethyltetradecahydro-
37	27.747	11417775	1.00	6S-2,3,8,8-Tetramethyltricyclo[5.2.2.0(1,6)]undec-2-ene
38	28.122	1379236	0.12	Stigmasta-3,5-dien-7-one
39	29.092	2086861	0.18	Stigmast-4-En-3-One

Table No. 9.24: The peak results of *S. nigrum* methanolic berries extract

References:

1. Abbas, K.H., Paul, R.N., Riley, R.T., Tanaka, T. & Shier, W.T. (1998). Ultra Structural Effects Of AAL-Toxin TA From The Fungus *Alternaria alternaria* on Black Nightshade (*Solanum Nigrum*) Leaf Disc and Correlation with Biochemical Measures of Toxicity. *Toxicon*. 36 (12); 1821-1832.

2. Abbasi, M.A., Raza, A., Riaz, T., Shahzadi, T., Aziz-ur-Rehman, Jahangir, M., *et. al.*, (2010). Investigation on the Volatile Constituents of *Juglans regia* and their *In Vitro* Anti-Oxidant Potential. *Proc. Pakistan Acad. Sci*. 47 (3); 137-141.

3. Ahmad, K., Mumtaz, H., Ashraf, M., Luqman, M., Ashraf, M.Y. & Khan, Z.I. (2007). Indigenous Vegetation of Soone Valley: At The Risk of Extinction. *Pakistan J. Bot*. 39 (3); 679-690.

4. Akhtar, M.S. & Muhammad, M. (1989). Evaluation of the Gastric Antiulcerogenic Effects of Solanum Species of Economic Importance. *Ann. Missouri Bot. Gard*. 75 (3); 8.

5. Akthar, M.S. & Munir, M. (1989). Evaluation of Antiulcerogenic Effect of *Solanum nigrum*, *Brassica oleracea* and *Ocimum basilicum* in Rats. *J. Ethnopharmacol*. 27, 163-172.

6. Al-Qirim, T., Zaidi, S.M., Shahwan, M., Shattat, G. & Banu, N. (2008). Effect of *Solanum nigrum* on Immobilization Stress Induced Anti-oxidant Defence Changes in Rat. *Res. J. Bio. Sci*. 3 (12); 1426-1429.

7. Edmonds, J.M. & Chweya, J.A. (1997). *Black Nightshades: Solanum nigrum L. and Related Species*. International Plant Genetic Resources Institute. Rome, Italy. 1-115.

8. Halliwell, B. & Gutteridge, J.M.C. (1985). *Free Radicals in Biology* and *Medicine*, Oxford University Press, Oxford, UK. 366-415.

9. Heo, K.S. & Lim, K.T. (2004). Anti-oxidative Effects of Glycoprotein Isolated from *Solanum nigrum L. J. Med. Food*. 7 (3); 349-357.

10. Herrera-Arellano, A., Jimenez-Ferrer, E., Vega-Pimentel, A.M., Martínez-Rivera, Mde, L., *et. al.*, (2004). Clinical and Mycological Evaluation of Therapeutic Effectiveness of *Solanum chrysotrichum* Standardized Extract on Patients with *Pityriasis capitis* (Dandruff). A Double Blind and Randomized Clinical Trial Controlled with Ketoconazole. *Planta Medica*, 70 (6); 483-488.

11. Hussain, A.O., Virmani, D. & Pople, S.P. (1992). *Dictionary of Indian Medicinal Plants.* Central Institute of Medicinal and Aromatic Plants, Lucknow, 35.

12. Jainu, M. & Devi, C.S.S. (2004). Anti-Oxidant Effect of Methanolic Extract of *Solanum nigrum* Berries on Aspirin Induced Gastric Mucosal Injury. *Indian J. Clin. Biochem.* 19, 65-70.

13. Jainu, M. & Devi, C.S.S. (2006). Antiulcerogenic and Ulcer Healing Effects of *Solanum nigrum* (L.) On Experimental Ulcer Models: Possible Mechanism for the Inhibition of Acid Formation. *J. Ethnopharmacol*. 104, 156-163.

14. Jamil, A., Shahid, M., Khan, M.M. & Ashraf, M. (2007). Screening of Some Medicinal Plants for Isolation of Antifungal Proteins and Peptides. *Pakistan J. Bot*. 39 (1); 211-221.

15. Khattak, J.Z.K., Anwar, Z., Aftab, S., *et. al.*, (2012). *Solenum nigrum* as Potent Therapy: A Review. *British Journal of Pharmacology and Toxicology*. 3 (4); 185-189.

16. Kirtikar, K.R. & Basu, B.D. (1935). *Indian Medicinal Plants*. 2nd Edn., Lalit Mohan Basu, Allahabad, Vol. 3.

17. Kumar, V.P., Shashidhara, S., Kumar, M.M. & Sridhara, B.Y. (2001). Cytoprotective Role of *Solanum nigrum* against Gentamicin-Induced Kidney Cell (Vero Cells) Damage *in Vitro*. *Fitoterapia*. 72, 481-486.

18. Miller, M.J., Sadowsak-Krowicka, H., Chotinaruemol, S., Kakkis, J.K. & Clark, D.A. (1993). Amelioration of Chronic Ileitis by Nitric Oxide Synthase Inhibition. *J. Pharmacol. Exp. Ther*. 264 (1); 11-16.

19. Nadkarni, K.M. (1976). *Indian Materia Medica*. 3rd Edn., Popular Prakashan, Bombay. 1, 1156.

20. Quattrocchi, U. (2000). *CRC World Dictionary of Plant Names*. Taylor and Francis. 4 R-Z. USA, 2058.

21. Ravi, V., Saleem, T.S.M., Maiti, P.P., Gauthaman, K. & Ramamurthy, J. (2009b). Phyto-Chemical and Pharmacological Evaluation of *Solanum nigrum* Linn. *Afr. J. Pharm. Pharmaco*. 3 (9); 454-457.

22. Ravi, V., Saleem, T.S.M., Patel, S.S., Ramamurthy, J. & Gauthaman, K. (2009a). Anti-Inflammatory Effect of Methanolic Extract of *Solanum nigrum* Linn Berries. *Int. J. Appl. Res. Natur. Prod*. 2 (2); 33-36.

23. Ruch, R.J., Cheng, S.J., & Klaunig, J.E. (1989). Prevention of Cytotoxicity and Inhibition of Intracellular Communication by Anti-Oxidant Catechins Isolated from Chinese Green Tea. *Carcinogenesis*. 10, 1003-1008.

24. Seithe, A. & Anderson, G.J. (1982). Hair Morphology and the Relationships of Species in Solanum Sect. Basarthrum, *Plant Syst. Evol*. 139, 229-256.

25. Singh, S.P., Raghavendra, K., Singh, R.K. & Subbarao, S.K., (2001). Studies on Larvicidal Properties of Leaf Extract of *Solanum nigrum* Linn. (Family Solanaceae). *Curr. Sci*. 81 (12); 1529-1530.

26. Solanum Phylogen, (2006). *Solanaceae Source. Natural History Museum*. Agricultural Research Service (13 April).
Http://Www.Nhm.Ac.Uk/Researchcuration/Research/Projects/Solanaceaesource/Taxonomy/Phylogeny/Index.Jsp,

27. Solanum, L. (2010). *Germplasm Resources Information Network*. United States Department of Agriculture.
Http://Www.Ars-Grin.Gov/Cgibin/Npgs/Html/Genus.Pl?11264.

28. Takhtajan, A. (1997). *Diversity and Classification of Flowering Plants*. Columbia University Press, New York.

29. Tirzitis, G. & Bartosz, G. (2010). Determination of Antiradical and Anti-Oxidant Activity: Basic Principles and New Insights. *Biochemica polonica*. 57 (1); 139-142.

30. Veseliaia, L.S. (1974). *Rod Solanum L.* In: Josifovia M. (Eds.), Flora S.R. Srbije. SANU, Beograd, 6, 85-94.

31. Waterhouse, A. (1999). *Folin–Ciocalteau Micro Method for Total Phenol in Wine.* Current Protocols in Food Analytical Chemistry. 299, 152-78.

32. Waterhouse, A. (2009). Folin–Ciocalteau Micro Method for Total Phenol in Wine. (<http://www.waterhouse.ucdavis.edu/phenol/folinmicro.htm>).