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Antioxidant activity in Solanum nigrum leaves and fruit aqueous (water) and organic extracts

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ABSTRACT :

Solanum nigrum Linn. (Sn) commonly known as Makoi from Solanaceae family. In the present study *S. nigrum* leaves and fruit are collected, air dried, weighed and extract prepared in different solvents (methanol, ethanol, water and petroleum ether). The maximum percentage yield was obtained in aqueous (water) extract with 48.02% and 45.72% in leaves and fruit extract respectively, whereas, lowest in petroleum ether fruits and leaves with 7.0% and 5.5% respectively. The phytochemicals analysis revealed the presence of alkaloids, flavonoids, phlobatanins, glycosides, saponins, steroids and tannins in leaves and fruit extract, whereas terpenoids in fruit extract. The crude extracts gave significant antioxidant activity by different antioxidant assays at varying concentration from 1000 µg/mL to 1.95 µg/mL. The maximum activity obtained in fruit aqueous (water) and ethanolic leaf extract with IC_{50} values of 33.4 ± 0.76 µg/ml and 31.0 ± 1.32 µg/ml by DPPH and Alkaline DMSO method, respectively. The minimum activity in fruit extract was obtained by hydroxyl radical method. The standard used was ascorbic acid with IC_{50} value of 30.7 ± 1.34 µg/ml and 33.06 ± 1.62 µg/ml by DPPH method and Alkaline DMSO method, respectively.

Keywords: *Solanum nigrum*, Alkaline DMSO, DPPH, antioxidant and nitric oxide

Introduction :

Solanum nigrum Linn. (Sn) commonly known as Makoi or Black Nightshade is a dicot weed in the Solanaceae family. The plant is widely distributed in tropical and temperate regions [1]. It is one of the known medicinal plant and it used by number of herbal medicines for number of diseases. In Ayurveda and Unani medicinal system as well as folk medicine uses this plant for the treatment of toothache, dermatitis, eczema, nephritis, urethritis, sore throat, leucorrhea and cancer. The phytochemicals known to be responsible for its antioxidant property include alkaloids, phenols, steroidal saponins and polysaccharides [2]. The plant reported to contain 188 chemicals and is rich in alkaloids and contains solasonine, solanine, solamargine and β-solamagrine, solasodinsolanidine (0.09-0.65%). β-solamagrine and solasodinsolanidine are also found in leaves, whereas solanine is found in all parts of the plant and its level increases with plant maturity, soil type and climate [3, 4]. The presence of phytochemicals specially phenolics responsible for antioxidant property in *Solanum nigrum* [5]. One of the phytochemical glycoprotein separated from ethanolic extract of fruit was reported to scavenge OH, O and DPPH radical and showed better scavenging results by deoxyribose assay as compared with standard ascorbic acid. Another compound separated from *S. nigrum*, lunasin peptide was found to chelate Fe2+ ion and block generation of hydroxyl radical [6]. The fruit methanolic extract demonstrated significant antioxidant property in heart tissue dose dependant [7]. Antioxidants are defined as molecules which, in small concentrations protect, and prevent or reduce the extent of oxidative destruction of bio-molecules called free radicals. The free radicals are molecules having unpaired electron in the outer orbit which have deleterious effect on human body formed by many physiological processes [8]. They are usually unstable and very reactive. Reactive oxygen species (ROS) include free radicals are superoxide, hydroxyl, peroxyl ($RO_2^{\bullet}$), alkoxyl ($RO^{\bullet}$), and hydroperoxyl ($HO_2^{\bullet}$) radicals, as well as two free radicals are Nitrogen dioxide (NO_2) and nitric oxide [9, 10]. The antioxidants were stored in the body or derived from natural products and they neutralized the free radicals produced during various body functions [11]. The mechanism of action of antioxidant is chain-breaking by which the primary anti-oxidant donates an electron to the free radical present in the system (e. g., lipid radical) forming a new radical, more stable than the initial one. Such primary anti-oxidants consist of compounds such as flavonoids, tocopherol and ascorbic acid [12]. A number of researcher had reported antioxidant property in *S. nigrum* by different antioxidant methods. The leaves of the plant reported potent antioxidant property [13, 14]. The antioxidant property varies due to geographical location and climate conditions, even the solvent in which extract prepared and methanolic extract gave maximum activity by different methods with 26.58 mg/100 g GAE) by DPPH method, 8.5 mM $FeSO_{4.7}H_2O$ by FRAP method [15]. The *S. nigrum* extract also reported to exhibited significant scavenging activity by hydroxyl radical scavenging assay [16]. In the present study *Solanum nigrum* leaves and fruit extract aqueous (water) and organic (methanolic, ethanolic and petroleum ether) extracts were examined for antioxidant parameters. As studied by previous researchers the plant is well known for its therapeutic properties.

Methodology :

The plant selected for the present study is *Solanum nigrum* also known as Makoi or blacknight shade. It is known for its medicinal properties. The plant fruits and leaves were collected, washed, dried and extract prepared in aqueous (water) and organic (methanol, ethanol and petroleum ether) solvents. The extract was weighed 10 g and soaked 100 ml solvent for 24 hours, filtered using muslin cloth and dried in hot air oven at temperature of 40 º C. The dried extract was weighed and percentage yield calculated, as well phytochemicals and antioxidant were determined.

Determination of extraction yield of plant extract (% yield)

The weight of dried plant part was calculated and its extract prepared and its percentage yield of the extract was calculated. The yield (% w/w) from all dried extracts was calculated by the formula given below.

$$\text{Yield (\%)} = \frac{W_2 - W_1}{W_0} \times 100$$

Where, W_2 is the weight of the extract and the container, W_1 weight of the container alone and W_0 the weight of the plant powder [17].

Phytochemical Analysis (Qualitative) of Plant Extract

The phytochemical analysis was carried out to determine the active ingredient of the aqueous (water) and organic extracts on the basis of color formation by qualitative methods. The methods followed are given below [18 – 22].

Alkaloids

The alkaloids were determined in plant extract by using Wagner's reagent. The Wagner's reagent was prepared by dissolving 2 g of iodine and 6 g of potassium iodide in 100 ml of distilled water. The crude extract 500 mg dissolved in 500 mL of methanol and dipped in water bath at 37° C for 20 min. The extract was then filtered off and allowed to cool, from that 2 mL was taken and treated with few drops of Wagner's reagent. A reddish brown coloured precipitate indicates the presence of alkaloids.

Anthraquinones (Borntrager's test)

The crude extract was weighed 0.5 g and boiled with 10% hydrochloric acid (HCl) for few minutes in a water bath, filtered and cooled. To the filtrate equal volume of chloroform (CHCl3) and few drops of 10% ammonia was added and allowed to heat. The presence of anthraquinones were indicated by appearance of pink rose colour.

Flavonoids

The crude extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min, filtered and mixed with 1 mL dilute ammonia solution to 4 mL filtrate. The formation of yellow colour indicates the presence of flavonoids.

Phlobatannins

The extract was boiled with 1% aqueous hydrochloric acid (HCl) to observe the deposition of red precipitate for the presence of phlobatannins.

Glycosides (Fehling's Test)

The crude extract was weighed 0.5 g and dissolved in 5 ml of methanol. To the 2 ml of extract, 10 ml of 50% hydrochloric acid (HCl) was added in a test tube and kept at boiling water bath for 30 minutes. To the above solution 5 ml of Fehling's solution was added and the mixture was boiled for 5 min to observe a brick red precipitate as an indication for the presence of glycosides

Saponins (Frothing Test)

The crude extract 0.2 g was mixed with 5 ml of distilled water and then heated to boil. Frothing (appearance of creamy mist of small bubbles) shows the presence of saponins.

Steroids (Salkowski Test)

The crude extract was dissolved in methanol and to it 5 drops of concentrated Sulfuric acid (H_2SO_4) was added. The formation of red colour indicated the presence of steroids.

Tannins (Ferric Chloride Test)

The crude extract was weighed to 0.5 g and dissolved in 10 ml of distilled water. The solution was filtered and to it ferric chloride was added, the presence of tannin was indicted by blue black precipitate.

Terpenoids (Salkowski Test)

The extract was weighed 0.2 g and mixed with 2 ml of chloroform and 3 ml of concentrated hydrochloric acid (HCl) carefully to form a layer. A reddish brown coloration of the interface formed indicated positive results for the presence of terpenoids.

Reducing Sugar

The crude extract was weighed 0.5 g in test tube and dissolved in 1 ml of distilled water and to it 2 to 8 drops of Fehling solution was added and boiled for few minutes. The brick red precipitate is formed indicated the presence of reducing sugar.

Anti-oxidant Assay

S. nigrum leaves and fruit aqueous (water) and organic extracts were evaluated for antioxidant property by different methods. The varying concentrations of extract used for the study from 1.95 µg/mL to 1000 µg/mL. The standard positive control used was ascorbic acid. The antioxidant activity was determined by four methods Alkaline DMSO, Nitric oxide, DPPH and Hydroxyl radical scavenging method.

Alkaline DMSO (dimethyl sulfoxide) Method

The superoxide radical scavenging was evaluated by alkaline DMSO method. The protocol of Kunchandy and Rao (1990) [23] with some changes was followed [24, 25]. The plant extract was prepared at different concentration in DMSO. The crude extract 0.3 ml was mixed with 0.1 ml of nitro blue tetrazolium (1 mg/ml in DMSO) and 1 ml of alkaline DMSO (1 ml of DMSO containing sodium hydroxide 5 mM in 0.1 ml of water) to give a final volume of 1.4 ml. After few minutes absorbance was measured at 560 nm spectrophotometrically and percentage inhibition was calculated. The formula given below.

$$\text{Percentage super oxide scavenging activity} = \frac{\text{Test absorbance - Control absorbance}}{\text{Test absorbance}} \times 100$$

Anti-oxidant Assay by Nitric Oxide Radial Inhibition Assay

The crude extract 1 ml was mixed with 1 ml phosphate buffer saline and 4 ml (10 mM) sodium nitroprusside, incubated for 150 min at 25 º C. After incubation, 0.5 ml of reaction mixture and 1 ml sulphanilic acid reagent (0.33% in 20% glacial acetic acid) were added and incubated again for 5 min at room temperature (for diazotization reaction). Then 1 ml N-(1-naphthyl) ethylenediamine dihydrochloride was added and kept in diffused light for 30 min and absorbance was measured at 540 nm [26].

$$\text{Scavenging activity (\%)} = \frac{\text{Absorbance of control – Absorbance of extract}}{\text{Absorbance of control}} \times 100$$

DPPH (2, 2 – Diphenyl – 1- Picryl Hydrazyl) Radical Scavenging Activity Method

DPPH radical scavenging activities of all the fractions were determined by the method of Blois (1958) [27] with some modification. The crude plant extracts were prepared at different concentrations (5 mg/ml to 20 mg/ml) in methanol, initially 10 µl of extracts was mixed with 200 µl of 100 mM DPPH (dissolved in methanol) and incubated for 30 min at 37° C under dark condition. Ascorbic acid was used as control, while methanol was used as blank. The DPPH radical scavenging activity was determined by measuring the absorbance at 490 nm spectrophotometrically [28]. The percentage inhibitions were calculated by the formula given below.

$$\text{Scavenging activity (\%)} = \frac{\text{Absorbance of control – Absorbance of extract)}}{\text{Absorbance of control}} \times 100$$

Scavenging of Hydroxyl Radical in the Deoxyribose Method

Scavenging of hydroxyl free radical was measured by the method of Halliwell and co-workers (1987) [29] with minor changes. The reaction mixture prepared containing deoxyribose (3mM) 0.2 ml, ferric chloride (0.1mM) 0.2 ml, ethylenediaminetetraacetic acid disodium salt (EDTA) (0.1 mM) 0.2 ml, ascorbic acid (0.1 mM) 0.2 ml and hydrogen peroxide (2 mM) 0.2 ml in phosphate buffer (pH, 7.4, 20 mM). To the reaction mixture was added 0.2 ml of various concentrations of the extract or standard in DMSO to give total volume of 1.2 ml. The solution was then incubated for 30 min at 37° C. After incubation, ice-cold trichloroacetic acid (0.2 ml, 15% w/v), and thiobarbituric acid (0.2 ml, 1% w/v) in 0.25 N hydrochloric acid were added, kept at boiling water bath for 30 min. The absorbance was measured at 532 nm after cooling of reaction mixture [30, 25]. The percentage inhibition values were calculated by the formula given below.

$$\text{Scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}} \times 100$$

Statistical analysis

The experiments done in the present study were performed thrice and mean value taken as well as standard deviation was also calculated. The results were given as mean ± standard deviation (SD). The mean, standard deviation and percentage reduction values were calculated in MS excel.

Results and Discussion :

Fruits and leaves of *Solanum nigrum* were procured from local market washed, air dried, crude extract prepared and percentage yield was calculated. The maximum yield was obtained was 48.02% and 45.72% in leaves and fruit aqueous extract respectively. Whereas, lowest yield obtained in petroleum ether extract 7.0 % and 5.5 % in fruits and leaves extract, respectively. Methanol and Ethanol both are polar solvent and maximum phytochemicals are separated in these solvents, whereas, petroleum ether is non – polar in nature but percentage yield indicates presence of some non – polar components in plant [31]. The percentage yield difference of extracts might be attributed to the difference in the solvent polarities used which also plays a key role in increasing the solubility of phytochemical compounds. The present result justified by the polarity effect of the solvents [32]. The percentage yield of other extracts are given in tabular form (Table -1). The results were justified by other study in which percentage yield obtained was maximum in water and methanol extract that is 41.63 and 36.52% respectively, whereas, petroleum ether gave least amount [31].

The quantitative phytochemicals analysis showed presence of alkaloids, flavonoids, phlobatanins, glycosides, saponins, steroids and tannins in leaves and fruit extract, whereas terpenoids in fruit extract. Other studies quantitative phytochemical analysis of *Solanum nigrum* in aqeous leaf extract showed highest concentration of phenol (38.17±0.33mg/100g), followed by terpenoid, flavonoids, tannins, cardiac glycosides and saponins in descending order. Alkaloids had the least concentration (12.61 ± 0.81mg/100g) [33 – 36]. The leaf extract of *Solanum nigrum* L of Dibrugarh district showed abundant amount of tannins in it and moderate amount of alkaloid, flavonoids, is reported rich in tannins and saponins [37]. The present study with the previous research done showed that *Solanum nigrum* is rich in phytochemicals flavonoids and alkaloids and these phytochemicals are responsible for its antioxidant property.

The anti-oxidant activity of leaves and fruit aqueous (water) and organic extracts at varying concentrations were determined and gave potent activity. The results were given in percentage inhibition values. The increase in percentage showed stronger inhibition and highest scavenging activity of the plant extract. The maximum activity by alkaline DMSO method in fruit extract was obtained in aqueous (water) extract and minimum in petroleum ether extract. The percentage inhibition values were found to be 81.2 ± 0.77 %, 76.0 ± 0.05 %, 77.4 ± 1.08 %, 54.6 ± 0.34 % and 85.1 ± 0.02 % in aqueous (water), methanolic, ethanolic, petroleum ether and ascorbic acid (standard) at the concentration of 1000 µg/mL, respectively. The leaves extract also showed potent activity with Alkaline DMSO method, maximum was found in methanolic extract and minimum in petroleum ether extract. The percentage inhibition values obtained was 82.2 ± 0.11 %, 81.5 ± 1.73 %, 79.0 ± 1.56 %, 60.1 ± 0.22 % and 85.1 ± 0.02 % in methanolic, ethanolic, aqueous (water), petroleum ether and ascorbic acid (standard) at the concentration of 1000 µg/mL respectively. The results illustrate 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} values were calculated which denotes 50 % scavenging activity of extract concentration. The maximum IC_{50} obtained in fruit aqueous (water), leaves ethanolic compared with standard (ascorbic acid) with values 122 ± 0.64 µg/mL, 31.0 ± 1.32 µg/mL and 33.06 ± 1.62 µg/mL, respectively. The superoxide radicals are highly reactive species and damages cellular constituents [38]. The enzyme Superoxide dismutase catalyses the dismutation of highly reactive superoxide anion to oxygen and hydrogen peroxide. Superoxide anion is the first reduction product of oxygen which is measured in terms of inhibition of generation of O_2 which reduces a dye nitro blue tetrazolium (NBT) used in the experiment and produces blue colour formazan measured at 590 nm by UV – VIS spectrophotometer [39]. It is reported that flavonoids scavenge superoxide anions and are effective antioxidants. The antioxidants inhibit NBT formation, decreases absorbance with antioxidants due to consumption of superoxide anion in reaction mixture. The crude extract of *S. nigrum* scavenges super oxide radical and thus inhibits formazan formation. The *S. nigrum* extract reported maximum superoxide radical scavenging activity at 120 µg/mL and percent inhibition 60.06 ± 4.20% with IC_{50} value of 57.82 µg/mL concentration, whereas, IC_{50} 9.65 µg/mL concentration in ascorbic acid used as standard [40]. In another study *S. nigrum* leaf extract significantly scavenged O_2 in concentration dependent manner. The IC_{50} values obtained was 417 µg/mL [41].

The antioxidant activity by nitric oxide method was also significant and fruit aqueous extract scavenging activity was equal to the standard (ascorbic acid) used in the study. The percentage inhibition values of fruit extract by nitric oxide method was found to be 75.2 ± 0.24 %, 72.1 ± 0.03 %, 71.3 ± 1.45 %, 44.1 ± 1.32 % and 79.4 ± 0.76 % in aqueous (water), methanolic, ethanolic, petroleum ether and ascorbic acid (standard) at the concentration of 1000 µg/mL, respectively. The scavenging activity by nitric oxide radical scavenging assay in leaf extract of *S. nigrum* was comparatively less than fruit extract and maximum found in methanolic extract. The percentage inhibition values were 71.9 ± 0.22 %, 69.5 ± 0.31 %, 67.2 ± 0.04 % and 79.4 ± 0.76 % in methanolic, ethanolic, aqueous (water) and ascorbic acid at the concentration of 1000 µg/mL, respectively. The IC_{50} values were calculated and maximum in ascorbic acid (standard) than ethanolic fruit and methanolic leaves extract at the concentration of 45.2 ± 0.22 µg/mL, 63.0 ± 1.56 µg/mL and 124 ± 0.91 µg/mL respectively. The nitric oxide is also unstable like superoxide ions under aerobic condition. The nitrates and nitrites are produced after reaction with O_2 in aerobic conditions and become stable and forms intermediate N_2O_4, NO_2 and N_3O_4. The antioxidant scavenges nitrous acid in presence of test compound and this is estimated by using Griess reagent and determined by its decrease in extent [42]. The *S. nigrum* leaf extract provide protection against oxidative damage induced by biomolecules as reported and scavenged 51.7% Nitric oxide radicals at the concentration of 500 µg/ml. The IC_{50} values obtained was 483 µg/ml and results were dose dependent [41]. As reported previously, *S. nigrum* ethanolic extracts of leaf, stem, fruit and stem exhibited high scavenging activities against nitric oxide radicals as compared with standards [43].

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 turns into a yellow coloured diphenyl picryl hydrazine. The degree of discoloration indicates the

radical-scavenging potential of the anti-oxidant [44]. The maximum antioxidant activity in fruit by DPPH method was observed in aqueous (water) extract and minimum in petroleum ether extract. The percentage inhibition values in fruit extract obtained was 78.1 ± 0.52 %, 76.4 ± 0.14 %, 76.0 ± 1.44 %, 52.1 ± 1.44 % and 80.4 ± 0.22 % in aqueous (water), methanolic, ethanolic, petroleum ether and ascorbic acid at the concentration of 1000 µg/mL, respectively. The *S. nigrum* leaf extract gave lesser scavenging activity in aqueous extract as compared to fruit extract. The maximum scavenging activity in leaves by DPPH method was found in methanolic extract. The percentage inhibition values obtained was 77.1 ± 0.05 %, 75.4 ± 1.34 %, 68.4 ± 0.04 %, 56.4 ± 1.88 % and 80.4 ± 0.22 % in methanolic, ethanolic, aqueous (water), petroleum ether and ascorbic acid at the concentration of 1000 µg/mL, respectively. The strongest antioxidant activity by DPPH method was found in aqueous (water) fruit extract with IC_{50} value of 33.4 ± 0.76 µg/mL equal to the standard used ascorbic acid with IC_{50} value of 30.7 ± 1.34 µg/mL. In leaves methanolic extract gave maximum activity as compared to other extracts with IC_{50} value of 67.5 ± 0.92 µg/mL. *S. nigrum* aqueous extract scavenges dose dependent DPPH radical and changes colour from purple coloured free radical to colourless α – α – diphenyl – β – picryl hydrazine. The extract scavenges 94.0 % at the concentration of 500 µg/mL and IC_{50} value of 165 µg/mL as reported antioxidant property due to presence of phenolic phytochemicals [41]. In other studies, maximum DPPH activity in *S. nigrum* was reported in methanol and ethanol extract with 73.16 ± 5.12 % at the concentration of 120 µg/mL and IC_{50} value of 81.02 µg/mL in ethanolic extract. The values are compared with the standard ascorbic acid with IC_{50} value of 11.98 µg/mL [40]. The standard ascorbic acid and rutin were reported as most potent and active antioxidants by DPPH method. It is reported that *S. nigrum* extracts were electron donors and convert free radicals in stable products by terminating the radical chain reaction [43]. The DPPH scavenging activity significantly affected on solvents used for extraction as methanol and ethanol are best solvent for polyphenol extraction and these two give more than 50 percent scavenging of DPPH radicals [45].

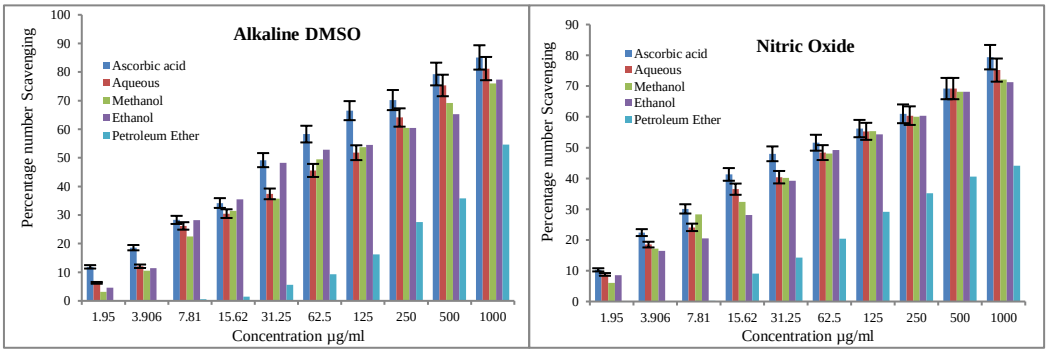
The percentage inhibition values by hydroxyl radical scavenging assay of *S. nigrum* leaf and fruit extract was found less than other scavenging assay. The maximum activity in fruit was found in ethanolic and minimum in petroleum ether. The percentage inhibition values obtained was 42.3 ± 0.91%, 40.1 ± 0.06 %, 31.4 ± 0.89 %, 22.1 ± 1.44 % and 55.4 ± 1.43 at the concentration of 1000 µg/mL in ethanolic, methanolic, aqueous (water), petroleum ether and ascorbic acid fruit extract, respectively. The results of scavenging activity by hydroxyl radical in *S. nigrum* leaf extract was found maximum in methanolic and minimum in petroleum ether extract. The percentage inhibition value of 49.4 ± 0.80 %, 45.7 ± 0.08, 28.3 ± 1.56, 24.5 ± 0.62% and 55.4 ± 1.43 % at the concentration of 1000 µg/mL in methanolic, ethanolic, aqueous (water), petroleum ether and ascorbic acid extract, respectively. The fruit and leaf extracts gave less activity than standard ascorbic acid used in the study by hydroxyl radical scavenging assay with IC_{50} value obtained was 560 ± 1.43 µg/mL. The other extracts gave lesser than 50 percent inhibition at highest concentration, therefore, no IC_{50} values obtained in the present study. The hydroxyl radical scavenging assay are dose dependant and in the present study comparatively less than other assay used. The other researchers had reported scavenging activity by hydroxyl radical assay. In one of the study the presence of solanine compound separated from *S. nigrum* demonstrated 60.22 percent inhibition at the concentration of 100 µg/mL [46].

The comparative chart of percentage inhibition values in different concentrations in *S. nigrum* fruit and leaves extract by Alkaline DMSO, Nitric oxide, DPPH and hydroxyl radical were given in graphical representation (Figure – 1 and 2). The comparative chart of IC_{50} value of fruit and leaves extract by different methods are given in tabular form (Table – 2 and 3).

A number of antioxidant components in plant are known and studied previously. Due to diversity in phytoconstituents one method is not accepted for analysis of antioxidant property and atleast two to three or more protocols are used for analysis of antioxidant potential of plant [47]. The variation is due to polarity, functional groups and chemical behaviour of various compounds present in plant. *S. nigrum* is rich in phytoconstituents and have different medicinal properties. The plant leaf and fruit extract were used for the analysis of antioxidant properties by different methods. All the antioxidant methods gave a promising results and antioxidant property obtained by them. The maximum and potent activity was obtained by Alkaline DMSO method and in fruit extracts. Methanol and ethanol extract was most potent due to presence of maximum phytoconstituents isolated in it, as well as both the solvent are polar in nature [47]. The minimum antioxidant activity was obtained in hydroxyl radical scavenging assay.

Percentage Yield (%)		
SOLVENTS	***Solanum nigrum* fruit**	***Solanum nigrum* leaves**
Petroleum ether	7.0	5.27
Water	45.72	48.02
Methanol	40.83	39.12
Ethanol	42.72	40.01

Table - 1: Percentage yield of *Solanum nigrum* fruit and leaves extracts in different solvent.



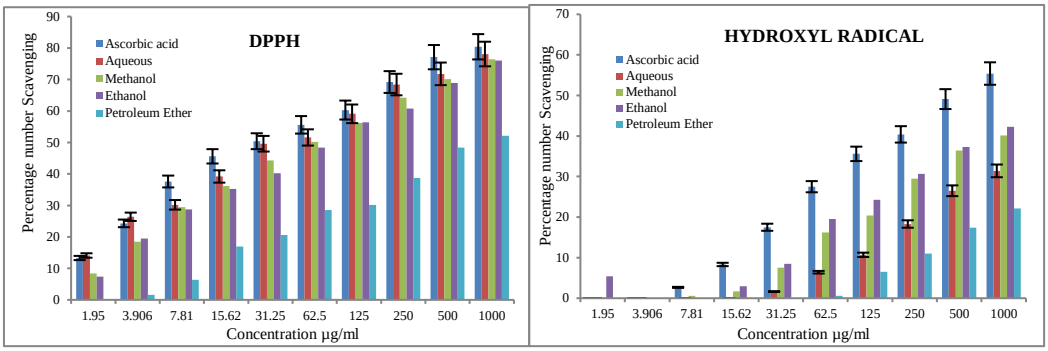


Figure 1: Graphical representation of percentage inhibition values of aqueous (water) and organic extracts of *Solanum nigrum* fruit compared with Ascorbic acid (standard) by Alkaline DMSO, Nitric oxide, DPPH and Hydroxyl radical method. Values are the average of triplicate experiments and represented as mean ± standard deviation. The concentration of extract and standard used were 1000 µg/mL to 1.95 µg/mL.

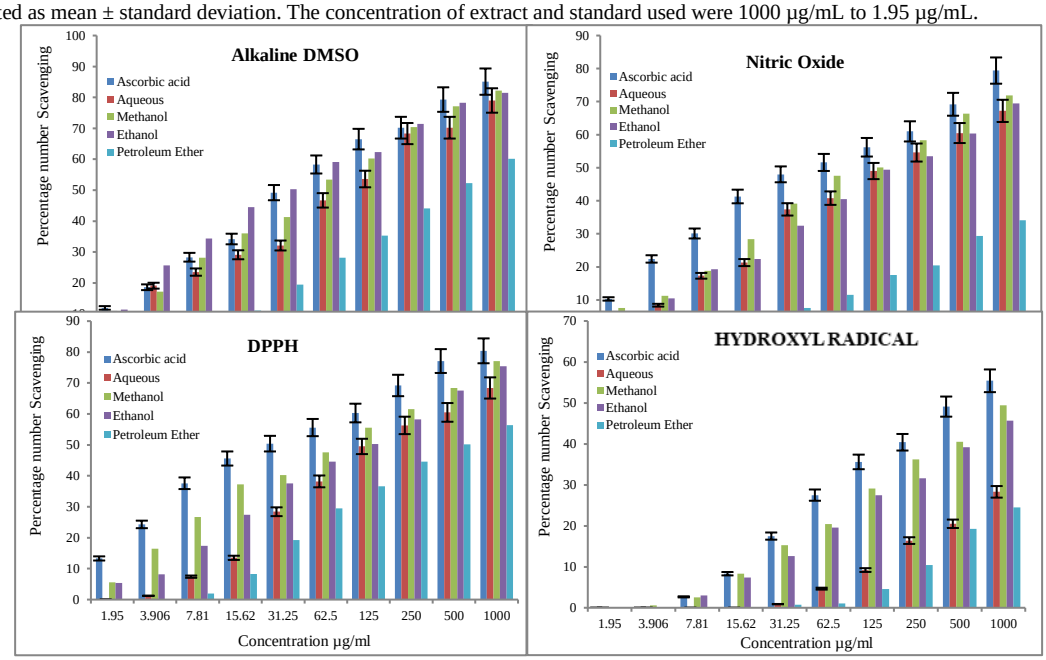


Figure 2: Graphical representation of percentage inhibition values of aqueous (water) and organic extracts of *Solanum nigrum* leaves compared with Ascorbic acid (standard) by Alkaline DMSO, Nitric oxide, DPPH and Hydroxyl radical method. Values are the average of triplicate experiments and represented as mean ± standard deviation. The concentration of extract and standard used were 1000 µg/mL to 1.95 µg/mL.

S. No.	***Solanum nigrum* extract and Standard**	**IC_{50} values ± SD (µg/ml) of different anti-oxidant assay**			
		Alkaline DMSO	**DPPH**	**Nitric oxide**	**Hydroxyl Radical**
1.	Methanolic	140 ± 1.76	65.8 ± 1.45	65.8 ± 0.23	-
2.	Ethanolic	153 ± 0.53	68.9 ± 0.32	63.0 ± 1.56	-
3.	Aqueous (water)	122 ± 0.64	33.4 ± 0.76	65.3 ± 1.09	-
4.	Petroleum ether	877 ± 1.93	850 ± 0.65	-	-
5.	Ascorbic Acid	33.06 ± 1.62	30.7 ± 1.34	45.2 ± 0.84	560 ± 1.43

Table – 2: Comparative chart of IC_{50} values of *Solanum nigrum* fruit extracts and Standard. The IC_{50} values of *Solanum nigrum* aqueous (water), methanolic, ethanolic and petroleum ether extracts. The standard used was Ascorbic acid. Unit for IC_{50} for all the activities are µg/ml. Data are expressed as mean ± SD (n=3).

S. No.	***Solanum nigrum* extract and Standard**	**IC_{50} values ± SD (µg/ml) of different anti-oxidant assay**			
		Alkaline DMSO	**DPPH**	**Nitric oxide**	**Hydroxyl Radical**

1.	Methanolic	58.2 ± 1.62	67.5 ± 0.92	124 ± 0.91	-
2.	Ethanolic	31.0 ± 1.32	120 ± 1.56	186 ± 0.65	-
3.	Aqueous (water)	71.5 ± 0.03	128 ± 0.33	130 ± 1.45	-
4.	Petroleum ether	670 ± 1.48	480 ± 0.32	-	-
5.	Ascorbic Acid	33.06 ± 1.62	30.7 ± 1.34	45.2 ± 0.84	560 ± 1.43

Table – 3: Comparative chart of IC_{50} values of *Solanum nigrum* leaves extracts and Standard. The IC_{50} values of *Solanum nigrum* aqueous (water), methanolic, ethanolic and petroleum ether extracts. The standard used was Ascorbic acid. Unit for IC_{50} for all the activities are µg/ml. Data are expressed as mean ± SD (n=3).

Conclusion :

Solanum nigrum plant is well known for its therapeutic properties in ethanomedicine. In the present study leaves and fruit extract were prepared in aqueous (water) and organic (methanol, ethanol and petroleum ether) solvents. The maximum solubility of compounds were found in aqueous extract with percentage inhibition values of 48.02% and 45.72 % in leaves and fruit extract, respectively. The lowest yield was obtained in petroleum ether extract 7.0 % and 5.5 % in fruits and leaves extract respectively. The plant showed for the presence of alkaloids, flavonoids, phlobatanins, glycosides, saponins, steroids and tannins in leaves and fruit extract, whereas terpenoids in fruit extract by quantitative phytochemicals analysis. The extracts were screened for antioxidant property and showed potent activity by different methods. The maximum activity obtained in fruit aqueous (water) extract with IC_{50} values obtained was 33.4 ± 0.76 µg/ml by DPPH method. The standard used was ascorbic acid with IC_{50} value of 30.7 ± 1.34 µg/ml by DPPH method. The minimum activity in fruit extract was obtained by hydroxyl radical method. The maximum activity in *S. nigrum* leaf was obtained in ethanolic extract with IC_{50} value of 31.0 ± 1.32 µg/ml by Alkaline DMSO method. The value was less than ascorbic acid (standard) with IC_{50} value of 33.06 ± 1.62 µg/ml by Alkaline DMSO method. The maximum activity by alkaline DMSO method in fruit extract was obtained in aqueous (water) extract with percentage inhibition values of 81.2 ± 0.77 µg/ml. The percentage inhibition values in Ascorbic acid was comparatively more than extracts and was found to be 85.1 ± 0.02 percent.

The plant possesses significant antioxidant property and maximum activity by DPPH and Alkaline DMSO method. The fruit aqueous (water), methanolic and ethanolic extract gave maximum activity, whereas petroleum ether gave minimum activity. The plant leaf extract gave maximum activity in methanolic and ethanolic extracts. The antioxidant activity may be due to presence of phenolic compounds present in it. The plant can be further studied for its compound and its therapeutic properties.

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