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Research article

In-vitro antioxidant activity of total phenolic and flavonoid content of Solanum nigrum: A comparative study

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ABSTRACT

In the traditional system of medicine, *Solanum nigrum* belonging to the family Solanaceae has been considered as a remedy for treating various ailments like epilepsy, gastric ulcers, etc., and is believed to have hepatoprotective activities. In this study, the total phenolic and flavonoid content of three extracts (Petroleum Ether, Ethyl acetate and Methanol) from the solanum *nigrum* leaves and their antioxidant activity was carried out by using spectrophotometric methods. Methanol extract with high absorbance exhibited the highest antioxidant activity containing the highest quantity of phenolics and flavonoids followed by Ethyl Acetate and Petroleum Ether. The presence of phenolic and flavonoid compounds indicated that these compounds can contribute to antioxidant activity. These active constituents alone or in combination with others may be responsible for the observed antioxidant activity. The findings indicated promising antioxidant activity of crude extracts of leaves needs further exploration for their effective use in both modern and traditional systems of medicines. The *Solanum nigrum leaf can* be regarded as a promising candidate for natural plant sources of antioxidants with higher values. A comparative study of the total phenolic and flavonoid contents extracted by the solvents, and their in-vitro antioxidant activity that could be an effective remedy in hepatoprotection was carried out in the study.

Keywords: Flavonoids, Phenols, in-vitro, solvents, Solanum nigrum.

Introduction

The family Solanaceae comprises of more than 1500 species and among them many species with economic importance are distributed cosmopolitantly [3]. *Solanum nigrum*belongs to the family Solanaceae and is known as Makoi in Bhopal, india. Locally *Solanum nigrum* is used as a traditional medicine for treating various ailments like hepatomegaly, edema, gonorrhea [1], diuretic, epilepsy, gastric ulcers, promotes menstrual discharge and applied on painful swellings, ulcers and abscess [2]. Natural antioxidants are of immense interest for scientists due to their health upgrading and hepatoprotective properties. Plants being good sources of phytochemicals like flavonoids, phenols, Carotenoids, glutathione, vitamin E, ascorbic acid etc. act as best antioxidants [2]. Antioxidants are the compounds which prolong or hamper the oxidation of other molecules by inhibiting either the inauguration or extension of oxidizing chain reaction [7]. Antioxidants thus safeguard from the oxidative damage and involve compounds that either eliminate or renew the impaired molecules. They hinder or obstruct the oxidation initiated by free radicals and their adequate intake is believed to provide protection against the disease. Many antioxidant enzymes like superoxide dismutase speeds up and glutathione peroxidase neutralizes the disease inducing free

radicals. Plants producing antioxidants on a large scale, restrain the hepatocarcinomic cell growth by impeding the formation of free radicals [**5**]. Reactive oxygen species (ROS) like the hydroxyl and superoxide radicals, hydrogen peroxide and singlet oxygen are produced by our body as offshoots of biological reactions [**14**]. In the present study, the, the TPC and TFC analysis of the different extracts of the *Solanum nigrum* leaf values was determined spectophotometrically and the comparative strength based upon of the polarity of various extracts was carried out.

Materials and Methods

Collection of the plant: The *Solanum nigrum* plants were collected from the local fields of Berasiya about 46 kilometers from Bhopal- the capital of Madhya Pradesh, India. The plant was identified by the famous taxonomist Dr. Shoukat Saeed Khan, Professor, Department of Botany, Saifiya Science College Bhopal, M.P, and preserved vide proper voucher number 1/Herbarium/Botany, in the herbarium of the Department of Botany, Motilal Vigyan Mahavidyalaya (MVM), Bhopal.

Preparation of the extract: The fresh leaves of the *Solanum nigrum* plants were first washed with tap water and then with distilled water, shade dried for 7 days, grinded by using electric grinder to form the fine powder and preserved. The dried powder (500gm) of leaves was successively Soxhlet extracted using Petroleum Ether, Ethyl Acetate and Methanol for 90 hours.

Materials required
Determination of Total Phenolic Content (TPC) by Spectrophotometer

Methodology

The quantity of total phenols in the extracts was determined by the Folin- Ciocalteu's reagent and the total phenolic content is expressed as mg/g gallic acid equivalent (GAE) [**27:3**]. FC reagent bears phosphomolybedic/ phosphotungstic acid complexes [**28**]. The concentrations of 10, 20, 30, 40 mg/ml of gallic acid were prepared in methanol. The concentration of 1mg/ml of plant extract was also prepared in methanol. To the test 0.5ml of each sample was added and mixed with 2.5ml of a 10 fold dilute Folin Ciocalteu reagent and 2ml of a 7.5% sodium carbonate which acts as a buffer to adjust PH value [**19**]. The test tubes were covered with parafilm, allowed to stand for 30 minutes at room temperature and the reading of absorbance was taken by a spectrophotometer at 760 nm. All the calculations were performed in triplicate. The Folin Ciocalteu reagent being sensitive to reducing compounds like polyphenols, upon reaction, they produce a blue color. The color produced was quantified spectrophotometrically [**17: 22:15**].

For the estimation of Total Phenol Content, line of regression from Gallic acid was used. From the standard curve of gallic acid, the line of regression was found to be

Y = 0.0025x + 0.0823

R2 = 0.0848 (Fig. A)

Y is the absorbance and x is the µgGAE/mg of the extract

Thus for the selected standard curve, the goodness of fit was found to be good by putting the absorbance of test sample (y

= absorbance) in the line of regression of above mentioned Gallic Acid.

Determination of Total Flavonoid Content (TFC) by Spectrophotometry

Methodology

The quantity of Total flavonoids in the extracts was estimated by Aluminium Chloride Assay through Colorimetry [**33, 34**]. Different plant extract concentrations were prepared (10- 100µg/ml) in methanol and also a test sample in methanol or any other of same polarity. An aliquot of 0.5ml of extracts were taken in different test tubes then 2ml of distilled water was added followed by the addition of 0.15ml of 5% sodium nitrate solution [5% NaNO2, W/V] and allowed to stand for 6 minutes. Later 0.15ml of 10% aluminium chloride (10% AlCl3) was added and incubated for 6 minutes, followed by the addition of 2ml of4% Sodium hydroxide solution (NaOH, 4% w/v) to the mixture and the final volume was made up to 5ml by adding distilled water, thoroughly mixed and allowed to stand for 15 minutes. After 15 minutes of incubation the mixture turns to pink, its absorbance was measured at 510nm using a colorimeter. Distilled water was used as a blank. The TFC was expressed in mg of Rutin Equivalents (RE) per gram of extract and was expressed as mg rutin/g dry weight (mg rutin/g DW), through the calibration curve of Rutin. All the determinations were carried out 3 times. The standard curve for different concentrations of the plant extract was prepared to find out the line of regression. The absorbance of test sample was put in the line of regression of standard curve of plant extract. Line of regression from rutin was used for the calculation of unknown flavonoid content which is expressed as µg/mg plant extract equivalent from standard curve of rutin.

$$\mathbf{Y = 0.0014x + 0.1071}$$

$$\mathbf{R^2 = 0.9437}$$

Antioxidant activity

DPPH Radical Scavenging Assay

Methodology

In DPPH assay, 0.1 mM DPPH solution (4mg/100ml) was prepared in methanol. Extract Samples were prepared to get concentration of 1mg/ml in methanol, various concentrations of sample solution is further diluted with methanol to 2ml than added 1ml of DPPH solution incubated at room temperature for 10 min absorbance was measured at 517 nm against blank [**16**]. The free radical scavenging activity was expressed as the percentage inhibition which was calculated by using the following formula:

Inhibition % = (A0 - A1 / A0) x 100

Where A0= Absorbance of the control
A1= Absorbance of the sample

The inhibition concentration (IC-50) value was determined from extrapolating the graph of % Inhibition versus the concentration of extract (using linear regression analysis), which is defined as the amount of antioxidant necessary to reduce the initial radical concentration by 50%. Lower the IC-50 value higher the antioxidant effects.

Superoxide radical scavenging activity

Principal

The principal behind this assay was the capability to inhibit reduction of nitro blue tetrazolium (NBT) in the NBT system [6]. For determination of superoxide dismutase activity, a method developed by Martinez et al., was used with a slight modification.

Methodology

Each 3 ml reaction mixture comprised of 50 mM sodium phosphate buffer, pH 7.8, 13 mM methionine, 2 mM riboflavin, 100 mM EDTA, NBT (75 mM) and 1 ml sample solution. The formation of blue color formazan was followed by perceptive the rise in absorbance after 10 min lighting from a fluorescent lamp at 560 nm. The whole reaction assembly was surrounded in a box, covered with aluminium foil. Tubes with reaction mixture were kept in the dark and served as blanks.

Super oxide scavenging activity (%) = (A0–A1)/A0×100, where A0 is absorption of control, A1 is absorption of tested extract solution.

Reducing Power assay

Principle: The basis of this method is the increase in the absorbance of the reaction mixture which indicates increase in the antioxidant activity. Substances with reducing power react with potassium ferricyanide (Fe3+) to form potassium ferrocyanides (Fe2+), which then react with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

Methodology

The reducing power was estimated by the method of Athukorala et al., [32]. An extract of 1ml was mixed with phosphate buffer measuring 2.5 ml (200 mM, pH 6.6), 2.5 ml (30mM) of potassium ferricyanide and was incubated for 20 minutes at 50°C. Add 2.5 ml of trichloroacetic acid (600 mM) to the mixture and centrifuge it for 10 minutes at 3000 rpm. To the upper 2.5 ml of solution add 2.5 ml of distilled water and 0.5ml of FeCl3 (6 mM) and at 700nm, the absorbance was measured. As a positive control, Ascorbic acid was used.

Antioxidant

Potassium ferricyanide + Ferric chloride → Potassium ferrocyanide + Ferrous chloride

1 mL of various concentrations of extract was mixed with 2.5 ml phosphate buffer solution (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The solution was properly mixed and placed in incubator for 20 min at 50ºC. After incubation, the resulting solution was cooled and 2.5 ml of 10%tri chloro acetic acid was added to reaction mixture, followed by centrifugation at 3000 rpm for 10 min. After centrifugation 2.5 ml of supernatant was mixed with equal volume of distilled water and finally 0.5 ml of 0.1% ferric chloride was added. The reaction mixture was shaken and kept at room temperature for 10 min. The absorbance was measured at 700 nm.

Results and Discussion

Total Phenolic Content

Polyphenols like flavonols, phenolic acids etc. are a distinct class of plant phenolic compoundsactingas free radical scavengers. The antioxidative nature of polyphenols is due to their highest reactivity as the polyphenol derived radical donates hydrogen or electron to stabilize and delocalize the unpaired electron and they have the ability to chelate metal ions [**20**]. They are powerful chelators of redox-active metal ions which prevent the transformation of hydroperoxides to reactive ox radicals by inactivating the chain reactions of free radicals [**21**]. Significant quantities of phenolics were found in the different extracts of *Solanum nigrum* leaves. Many studies revealed that the yield of phenols extracted depend upon the polarity of the solvent used, indicating that high polarity solvents being used are best for extraction [**19**].

For the phenolic extraction, the efficacy of solvents used was found to be in the order: Methanol > Ethyl acetate > Petroleum ether (Table V). It was noticed that on using solvents with high polarity, the yield of TPC also increased and the largest quantity was seen in Methanol extract while the smallest quantity was found in Petroleum ether extract (Table IV, II). The quantitative analysis of TPC of extracts, indicated that the Methanol extract contains largest amount of TPC (135.16µgGAE/mg), followed by Ethyl acetate extract (68.50µgGAE/mg) and Petroleum Ether (13.00 µg GAE/mg). The quantitative analysis of TPC was found to be in the order of Methanolic Extract> Ethyl acetate Extract> Petroleum Ether Extract (ME > EAE> PEE) (Table V).

Table 1: Absorbance v/s concentration of Gallic Acid

S. No.	Concentration of Gallic acid(µg/ml)	Absorbance
1	10	0.121
2	20	0.194
3	30	0.243
4	40	0.281
5	50	0.33

Values are expressed as Mean±SD

Figure 1: A Standard curve of Gallic acid

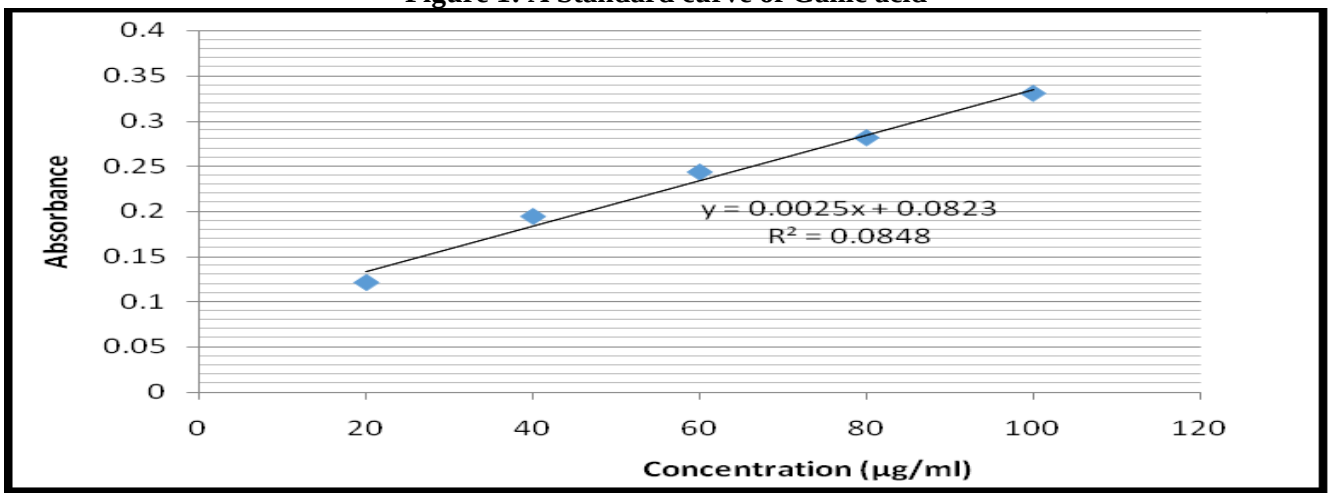


Table ll: Total Phenolic content in petroleuam ether extract

S. No.	Absorbance	Concentration	Total Phenolic content in mg/g equivalent of Gallic acid
1	0.111	1mg/ml	13.00
2	0.108	1mg/ml	
3	0.105	1mg/ml	
		Mean±SD	**13.00**

Values are expressed as Mean± SD µg of Gallic acid equivalent per milligram of dry weight (µg GAE/mg) of the extract. Determination was performed in triplicates.

Table III: Total Phenolic content in ethyl acetate extract

S. No.	Absorbance	Concentration	Total Phenolic content in mg/g equivalent of Gallic acid
1	0.219	1mg/ml	68.50
2	0.218	1mg/ml	
3	0.22	1mg/ml	
		Mean±SD	**68.50**

Values are expressed as Mean± SD µg of Gallic acid equivalent per milligram of dry weight (µg GAE/mg) of the extract. Determination was performed in triplicates.

Table IV: Total Phenolic content in Methanolic extract

S. No.	Absorbance	Concentration	Total Phenolic content in mg/g equivalent of Gallic acid
1	0.353	1mg/ml	135.16
2	0.352	1mg/ml	
3	0.352	1mg/ml	
		Mean±SD	**7.50**

Values are expressed as Mean± SD µg of Gallic acid equivalent per milligram of dry weight (µg GAE/mg) of the extract. Determination was performed in triplicates.

Table V: Comparison of Total Phenolic Content in Petroleum Ether, Chloroform and Ethyl Acetate (GAE)

S. No	Extract	Conc. Of extract	TPC Content g/mg equiv. To Gallic acid
01	Petroleum Ether	1mg/ml	13.00
02	Ethyl Acetate	1mg/ml	68.50
03	Methanolic	1mg/ml	135.16
	Mean SD		72.22

Total Flavonoid content

Flavonoids are a group of secondary plant metabolites acting as effective antioxidants and chelators. This is due to their ability of free radical scavenging, chelating metal ions like iron and copper and obstructing enzymes producing free radicals [5]. The structure and the substitution design of their hydroxyl groups make them active antioxidants [26]. Because of their scavenging abillity, flavonoids are known as Reactive Oxygen Species (ROS) Antioxidants delay the process of oxidation by restraining the polymerisation chain reaction begun by free radicals [7]. Phytochemicals being the natural antioxidants have the capacity to relieve the fatal effects of the diseases affiliated to oxidative stress [23]. Phenols and flavonoids are reported to be strong antioxidants because of their hydroxyl groups (10). Same solvents were used in the estimation of TFC as used in TPC. The flavonoids were extracted most efficiently in methanol compared to other solvents used. The results of the study coincide with that of Spigno et al.,(2007) [24] who implied that polar solvents extract flavonoids very much, because of the increase in polarity, flavonoids conjugate with hydroxyl groups through glycosides, thus increasing their solubility in polar solvents [13]. The quantitative estimation of TFC indicates that methanol extracted it in largest quantity (230.66 µg RE /mg) followed by ethyl acetate (91.33 µg RE /mg) and least by Petroleum Ether (11.00 µg RE /mg). (TableXIV). The pattern of the quantitative estimation of TFC was found as Methanol extract > Ethyl Acetate > Petroleum Ether (ME > EAE >PEE) (Table X).

Total Flavonoid Content

Table VI: Absorbance v/s concentration of Rutin

S. No.	Concentration (ug/ml)	Absorbance
1	10	0.142
2	20	0.163
3	30	0.181
4	40	0.206
5	50	0.259

Values are expressed as Mean ±SD

Figure 2: II Standard Curve of Rutin

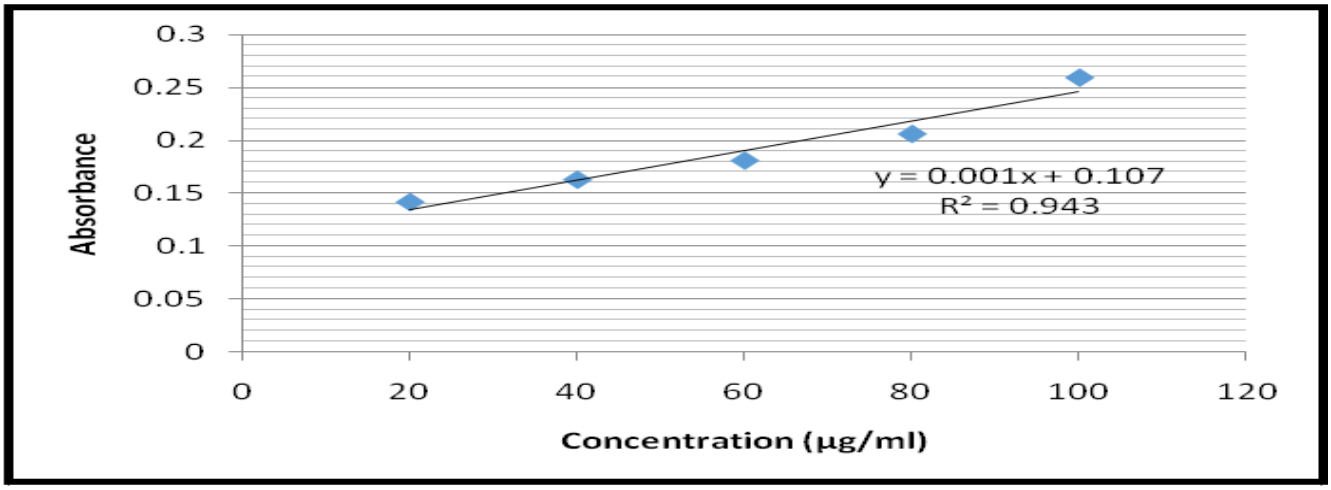


Table VII: Total Flavonoid content in Petroleum Ether Extract(PEE)

S. No.	Absorbance	Concentration	Total Flavonoid content in mg/g equivalent of Rutin
1	0.118	1mg/ml	11.00
2	0.119	1mg/ml	
3	0.117	1mg/ml	
		Mean±SD	11.00

Values are expressed as MEAN± SDµg of Rutin equivalent per milligram of dry weight (µg RE/mg) of the extract. Determination was performed in Triplicates.

Table VIII: Total Flavonoid content in Ethyl Acetate Extract (EAE)

S. No.	Absorbance	Concentration	Total Flavonoid content in mg/g equivalent of Rutin
1	0.207	1mg/ml	91.33
2	0.192	1mg/ml	
3	0.196	1mg/ml	
		Mean±SD	91.33

Values are expressed as MEAN± SDµg of Rutin equivalent per milligram of dry weight (µg RE/mg) of the extract. Determination was performed in Triplicates.

Table IX: Total Flavonoid content in methanolic extract (ME)

S. No.	Absorbance	Concentration	Total Flavonoid content in mg/g equivalent of Rutin
1	0.337	1mg/ml	230.66
2	0.337	1mg/ml	
3	0.339	1mg/ml	
		Mean±SD	230.66

Values are expressed as MEAN± SDµg of Rutin equivalent per milligram of dry weight (µg RE/mg) of the extract. Determination was performed in Triplicates.

Table X: Comparison of flavonoid quantity extracted in Petroleum Ether, Ethyl Acetate and Methanol

S.No.	Extract	Conc. of extract	Total Flavonoid contentµg/mg equiv. To Rutin
01	Petroleum ether	1mg/ml	11.00
02	Ethyl acetate	1mg/ml	91.33
03	Methanol	1mg/ml	230.66
		MEAN±SD	110.99

Values are expressed as MEAN± SDµg of Rutin equivalent per milligram of dry weight (µg RE/mg) of the extract. Determination was performed in Triplicates.

Comparison of Total Phenolic and Flavonoid contents of Extracts

The Table (XI) representing the comparison of the Total Phenolic and Total Flavonoid contents in Petroleum Ether, Ethyl Acetate and Methanol Extract. The study revealed that Petroleum ether extracted much quantity of TPC than TFC (TPCPE > TFCPE), TPC quantity of Ethyl acetate extract was lower than its TFC (TPCEA < TFCEA) and TPC of Methanol extract was found to be lower than its TFC (TPCME < TFCME).

Table XI: Comparison of Total Phenolic and Total Flavonoid content of Petroleum Ether, Ethyl Acetate and Methanol extract.

S.No.	Extract	Total phenolic content µg/mg equiv. to gallic acid	Total Flavonoid content µg/mg equiv. to Rutin
01	Petroleum Ether	13.00	11.00
02	Ethyl Acetate	68.50	91.33
03	Methanol	135.16	230.66
	MEAN ± SD	72.22	110.99

Values are expressed as MEAN ± SD µg of Gallic acid equivalent per milligram of dry weight (µgGAE/mg) of the extract and MEAN ± SD µg of Rutin equivalent per milligram of dry weight (µg RE/mg) of the extract. Determination was performed in triplicates.

DPPH radical scavenging activity of *Solanum nigrum* Leaf

DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method forming a violet solution in ethanol is an antioxidant assay which depends on electron transfer. The free radical produces colorless ethanol solution on reduction in the presence of an antioxidant. This assay helps us to calculate antioxidants by spectrophotometer easily and rapidly [**13**]. The DPPH assay depends on the potential of an antioxidant to donate an electron or hydrogen radical to DPPH radical- an inert free radical bearing deep violet color. DPPH radical is reduced to its parallel hydrazine, DPPH-H shape on pairing with an unmatched electron when a free radical scavenger of antioxidant is present [18] and the color of the solution changes from deep violet to light yellow. The fall in absorbance is directly proportional to the antioxidant concentration and is analyzed spectrophotometrically [**11**]. The Table (xii) indicates that on increasing the concentration of ascorbic acid(control) from 10, 15, 20, 25 and 30 µg/ml, the values of absorbance get decreased in the order of 0.467 > 0.388 > 0.332 > 0.251 > 0.211 and the values of % inhibition increased in the order of 49.4041< 57.9632 < 64.0303 < 72.8061 < 77.1398 respectively. The table (xiii) depicting the radical scavenging activity of petroleum ether extract shows that on increasing the concentrations of the sample from 20, 40, 60, 80 and 100 µg/ml, the absorbance values are decreased in the pattern of 0.634 > 0.545 > 0.477 > 0.403 > 0.348 and also the values of inhibition % significantly increases in the order from 31.3109 < 40.9534 < 48.3207 < 56.338 < 62.2969 respectively. The table (xiv) representing the DPPH radical scavenging activity of Ethyl

Acetate extract describes that on increasing the concentrations of the sample from 20, 40, 60, 80 and 100 µg/ml, the absorbance values are decreased in the order of 0.507 > 0.456 > 0.401 > 0.385 > 0.258 and the values of inhibition % significantly increased in the order from 45.0704 < 50.5959 < 56.5547 < 58.2882 < 72.0477 respectively. The table (xv) representing the DPPH radical scavenging activity of Methanol extract delineates that on increasing the concentrations of the sample from 20, 40, 60, 80 and 100 µg/ml, the absorbance values of the samples are decreased in the order of 0.473 > 0.368 > 0.329 > 0.245 > 0.203 and the values of inhibition percentage largely increases in the order from 48.7541 < 60.13 < 64.3554 < 73.4561 < 78.0065 respectively.

The values of the tables conclude that on increasing the concentration of the extract samples, their value of asorbance decreases and the values of % inhibition increases. The present study depicts that the values of absorbance of the Petroleum Ether, Ethyl Acetate and Methanol are inversely proportional to that of the values of their % inhibition and coincides with the study carried out by [**11**], stating that the fall in absorbance is directly proportional to the antioxidant concentration [**11**]. Therefore, the *Solanum nigrum* leaf extracts showed significant antioxidant activities close to that of ascorbic acid. Also among all the three leaf extracts used i.e., Petroleum Ether, Ethyl Acetate and Methanol, Methanol extract showed the highest values of inhibition % on the DPPH, depicting that it is a strong antioxidant than Ethyl Acetate and Petroleum Ether and their efficacy as antioxidants can be expressed as Methanol extract of leaf > Ethyl Acetate extract of leaf > Petroleum Ether extract of leaf (MEL < EAL < PEEL) (Table XVII).

Table XII: DPPH Radical scavenging activity of Ascorbic acid

Ascorbic acid			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	10(µg/ml)	0.467	49.4041
2.	15(µg/ml)	0.388	57.9632
3.	20(µg/ml)	0.332	64.0303
4.	25(µg/ml)	0.251	72.8061
5.	30(µg/ml)	0.211	77.1398
IC_{50}		**9.85**	

Table XIII: DPPH Radical scavenging activity of Pet ether extract of *Solanum nigrum*

Pet ether extract of *Solanum nigrum*			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	20 (µg/ml)	0.634	31.3109
2.	40(µg/ml)	0.545	40.9534
3.	60(µg/ml)	0.477	48.3207
4.	80(µg/ml)	0.403	56.338

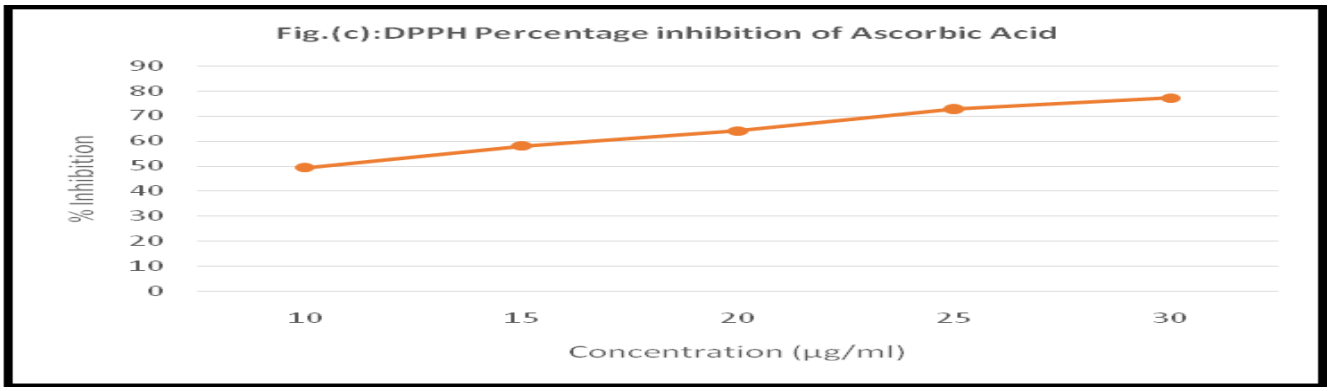
5.	100(µg/ml)	0.348	62.2969
IC_{50}		**65.72**	

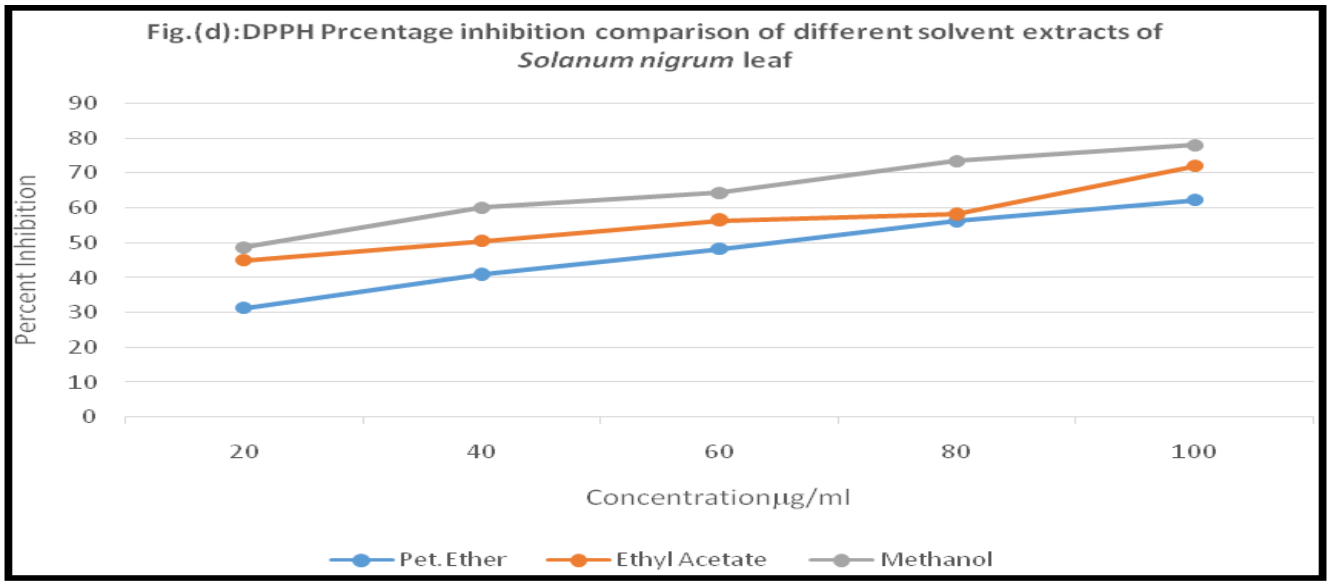
Table XIV: DPPH Radical scavenging activity of ethyl acetate extract of *Solanum nigrum*

Ethyl acetate extract of *Solanum nigrum*			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	20 (µg/ml)	0.507	45.0704
2.	40(µg/ml)	0.456	50.5959
3.	60(µg/ml)	0.401	56.5547
4.	80(µg/ml)	0.385	58.2882
5.	100(µg/ml)	0.258	72.0477
IC_{50}		**38.92**	

Table XV: DPPH Radical scavenging activity of Methanolic extract of *Solanum nigrum*

Methanolic extract of Solanum nigrum			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	20 (µg/ml)	0.473	48.7541
2.	40(µg/ml)	0.368	60.13
3.	60(µg/ml)	0.329	64.3554
4.	80(µg/ml)	0.245	73.4561
5.	100(µg/ml)	0.203	78.0065
IC_{50}		**20.23**	





TableXVI: Absorbance Comparison of Petroleum Ether, Ethyl Acetate and Methanol extracts of *Solanum nigrum* leaf.

S.No.	Con. µg/ml	PetroleumEther	Ethyl Acetate	Methanol
1	20	0.634	0.507	0.473
2	40	0.545	0.456	0.368
3	60	0.477	0.401	0.329
4	80	0.403	0.385	0.245
5	100	0.348	0.258	0.203

Table XVII: Comparison of percentage inhibition of Petroleum Ether, Ethyl Acetate and Methanol extracts of *Solanum nigrum* leaf.

S.No	Con.µg/ml	Petroleum Ether	Ethyl Acetate	Methanol
1	20	31.3109	45.0704	48.7541
2	40	40.9534	50.5959	60.13
3	60	48.3207	56.5547	64.3554
4	80	56.338	58.2882	73.4561
5	100	62.2969	72.0477	78.0065

Superoxide scavenging activity of Solanum nigrum leaf

Superoxide anion being a weak oxidant results in the production of strong and fatal hydroxyl radicals and oxygen in excited state, both create oxidative stress (12). Superoxide anions being most toxic species are produced by different biological reactions. In the present study the superoxide scavenging activity of the Petroleum Ether, Ethyl Acetate and Methanol Extracts of the *Solanum nigrum* leaf were studied. (Table XIX,XX,XXI) representing the superoxide scavenging activity of ascorbic acid (control), depicts that as we go on increasing the concentration of the sample from 10, 15, 20, 25, 30µg/ml, its absorbance decreases in the order from 0.489 > 0.457 > 0.419 > 0.368 > 0.334 and its % inhibition increases in the order as 51.63205 < 54.79723 < 58.55589 <63.6004 < 66.9634. In (Table XXII), representing the superoxide scavenging activity of Petroleum Ether extract of the *Solanum nigrum* leaf, as we increase the concentration of the sample from 20, 40, 60, 80, 100µg/ml, its absorbance goes on decreasing from 0.614 > 0.549 > 0.465 > 0.398 > 0.291 and inhibition % increases from 32.26805 < 45.69733 < 54.00593 < 60.63304 <71.21662. Similarly in Table(XX), as we increase the concentration of Ethyl

Acetate extract, the absorbance is found in the order of 0.519 > 0.457 > 0.419 > 0.368 > 0.334, and the % inhibition is found in the order of 48.66469 < 54.79723 < 58.55589 < 63.6004 < 66.9634. Also in the (Table XXI), as we increase the concentration of the sample of the Methanolic Extract, the absorbance decreases from 0.489 < 0.444 < 0.386 < 0.290 < 0.237 and its % inhibition increases from 51.63205 < 56.08309 <61.81998 < 71.31553 < 76.55785. After comparing the values of the tables (Table XXII) it was found that the concentration of the samples is inversely proportional to their absorbance, and directly proportional to their % inhibition. The values of all the three samples show a decrease in absorbance and an increase in % inhibition as shown by the control sample, also it can be concluded that the Methanolic extract of the plant has the best superoxide scavenging activity, as it shows the lowest values of absorbance and highest values of % inhibition compared to that of the Ethyl Acetate and Petroleum Ether extracts. The efficiency of the three extracts can be represented in the increasing order as Petroleum Ether Extract (PEE) < Ethyl Acetate Extract (EAE) < Methanol Extract (ME).

Table XVIII: Superoxide scavenging activity of Ascorbic acid

S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	10(µg/ml)	0.489	51.63205
2.	15(µg/ml)	0.457	54.79723
3.	20(µg/ml)	0.419	58.55589
4.	25(µg/ml)	0.368	63.6004
5.	30(µg/ml)	0.334	66.9634
IC_{50}		**8.466**	

Table XIX: Superoxide scavenging activity of Pet ether extract of *Solanum nigrum* leaf

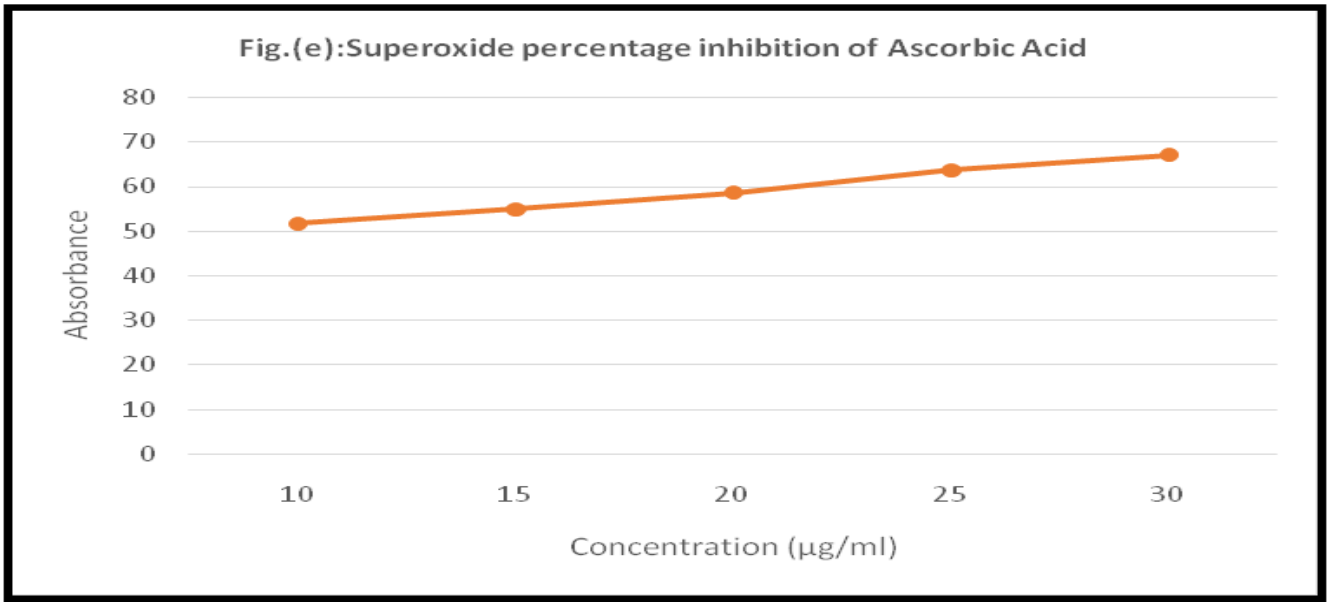
Pet ether extract of *Solanum nigrum*			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	20 (µg/ml)	0.614	39.26805
2.	40(µg/ml)	0.549	45.69733
3.	60(µg/ml)	0.465	54.00593
4.	80(µg/ml)	0.398	60.63304
5.	100(µg/ml)	0.291	71.21662
IC_{50}		**49.46**	

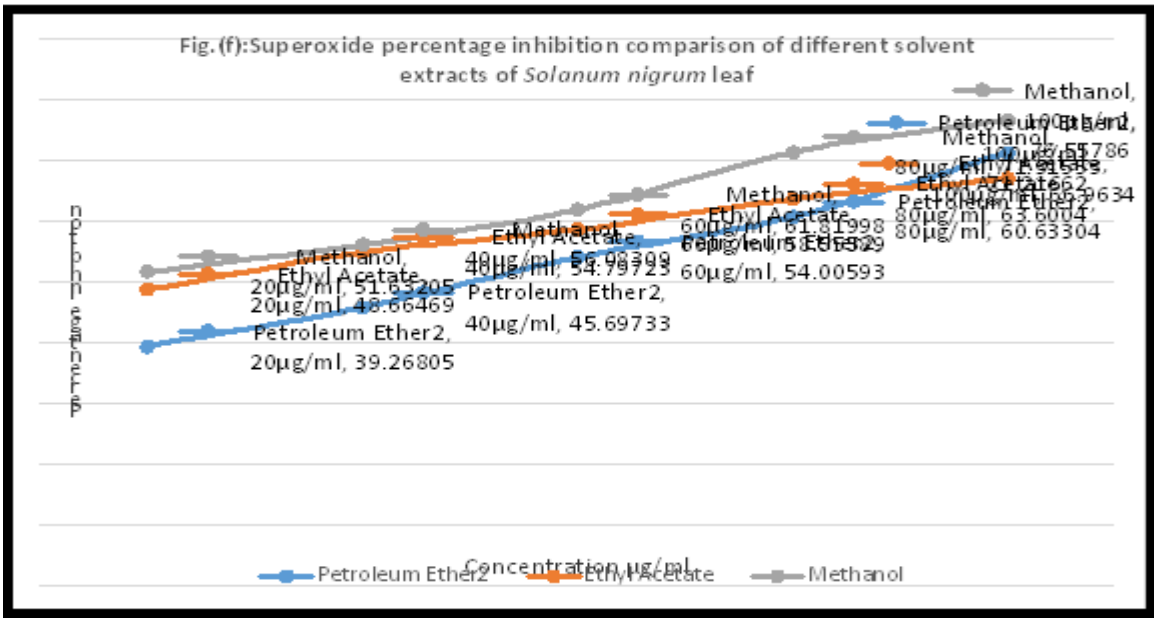
Table XX: Superoxide scavenging activity of Ethyl acetate extract of *Solanum nigrum*			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	20 (µg/ml)	0.519	48.66469
2.	40(µg/ml)	0.457	54.79723
3.	60(µg/ml)	0.419	58.55589
4.	80(µg/ml)	0.368	63.6004
5.	100(µg/ml)	0.334	66.9634
IC_{50}		**22.51**	

Table XXI: Superoxide scavenging activity of Methanolic extract of *Solanum nigrum* leaf			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	20 (µg/ml)	0.489	51.63205
2.	40(µg/ml)	0.444	56.08309
3.	60(µg/ml)	0.386	61.81998
4.	80(µg/ml)	0.29	71.31553
5.	100(µg/ml)	0.237	76.55786
IC_{50}		**18.61**	

Table XXI: Table representing comparative absorbance of extracts of Petroleum Ether, Ethyl Acetate and Methanol against their concentration.

S.No.	Concentrationµg/ml	Petroleum Etherµg/ml	Ethyl Acetateµg/ml	Methanolµg/ml
1.	20	0.614	0..519	0.489
2.	40	0.549	0.457	0.444
3.	60	0.465	0.419	0.386
4.	80	0.398	0.368	0.290
5.	100	0.291	0.334	0.237





Discussion

Reducing power being related to antioxidant activity may thus act as its important indicator [15]. Compounds with reducing potential signify that they can reduce the oxidized intermediates and act as primary and secondary antioxidants by donating the electrons [29]. The reducing power assay is practiced to find out the electron donating ability of an antioxidant [30]. The potential of leaf extracts to reduce Fe^{3+} to Fe^{2+} was found in this assay. Antioxidants carry out the reduction of Fe^{3+} to Fe^{2+}

by altering the solution into different shades, based on the reducing potential of the compounds [**8**]. Although powerful reducing agents produce Perl's Prussian blue color absorbed at 700nm. In the present study, Ascorbic Acid has been used as a control. (Table XXIII) and (Fig. e) showed the increase in the concentration of ascorbic acid versus the value of its absorbance which also showed a gradual increase in the reducing power as its concentration goes on increasing. Table (xxiv) and Fig.(f) depicting the relative comparison of the increasing concentrations (20, 40, 60, 80, 100µg) of the extracts of Methanolic Extract (ME), Ethyl Acetate Extract(EAE), Petroleum Ether Extract (PEE) to that of their value of absorbance. It was found that as the concentration of the extracts goes on increasing from 20, 40, 60, 80, 100µg, the values of their absorbance also increase in the ascending order from 0.067 < 0.08 < 0.108 < 0.13 < 0.147 in Methanolic Extract. In Ethyl Acetate, the absorbance values accelerated from 0.054 < 0.067 < 0.094 < 0.118 < 0.136 and in Petroleum Ether, these values enhanced in the increasing order from 0.031 < 0.04 < 0.058 < 0.082 < 0.098. Petroleum Ether Extract showed less degree of Fe^{3+} reduction than Ethyl Acetate Extract and Methanolic Extracts. The present study depicts that the increase in the concentration of the extracts of the *Solanum nigrum* leaf extracts, increases the absorbance values of all the extracts used, thereby reiterating that the concentration of the sample is directly proportional to the values of absorbance and the *Solanum nigrum* leaf extract used has a strong antioxidant activity. Also it was found that among all the extracts used, the Methanolic Extract used showed highest values of absorbance and is expressed in the increasing order of asorbance as Petroleum Ether Extract (PEE) < Ethyl Acetate Extract (EAE) Methanolic Extract (ME), also reducing power of the extracts used was found to be in the increasing order as Petroleum Ether Extract (PEE) < Ethyl Acetate Extract (EAE) Methanolic Extract (ME). The reducing power of a substance is reportedly because of their ability to donate hydrogen (25). Thus, Methanolic extract may contain large amount of reductions as compared to Ethyl Acetate and Petroleum Ether extract thus it may function as most efficient electron donors than Ethyl acetate and Petroleum ether extracts and could combine with free radicals to change them into more stable products to check the free radical chain reactions.

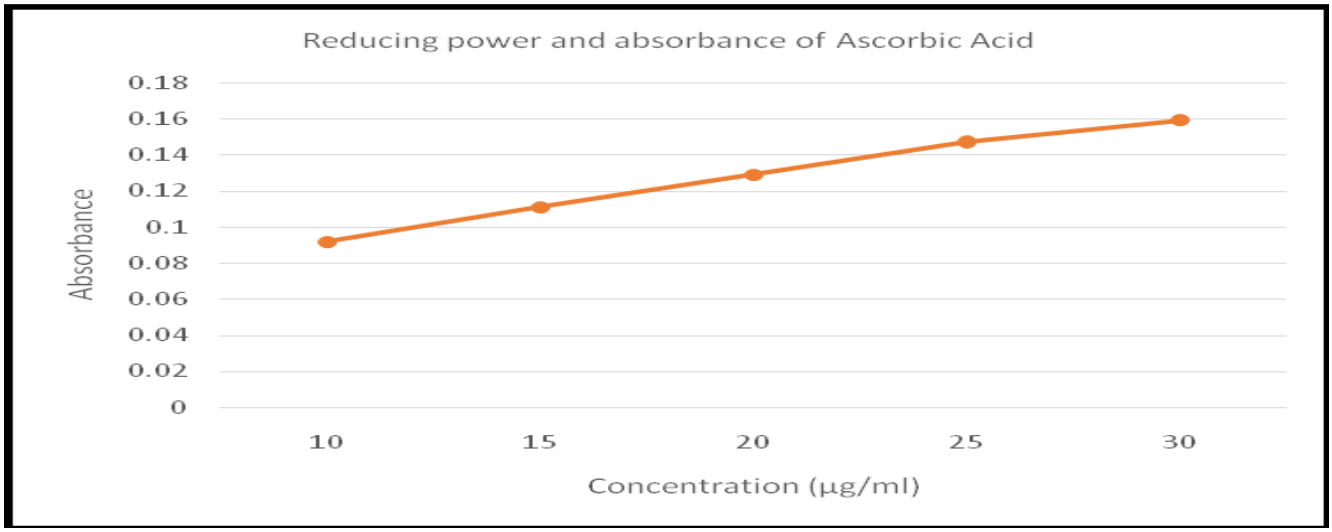
Table XXIII: Reducing Power Assay of Ascorbic acid

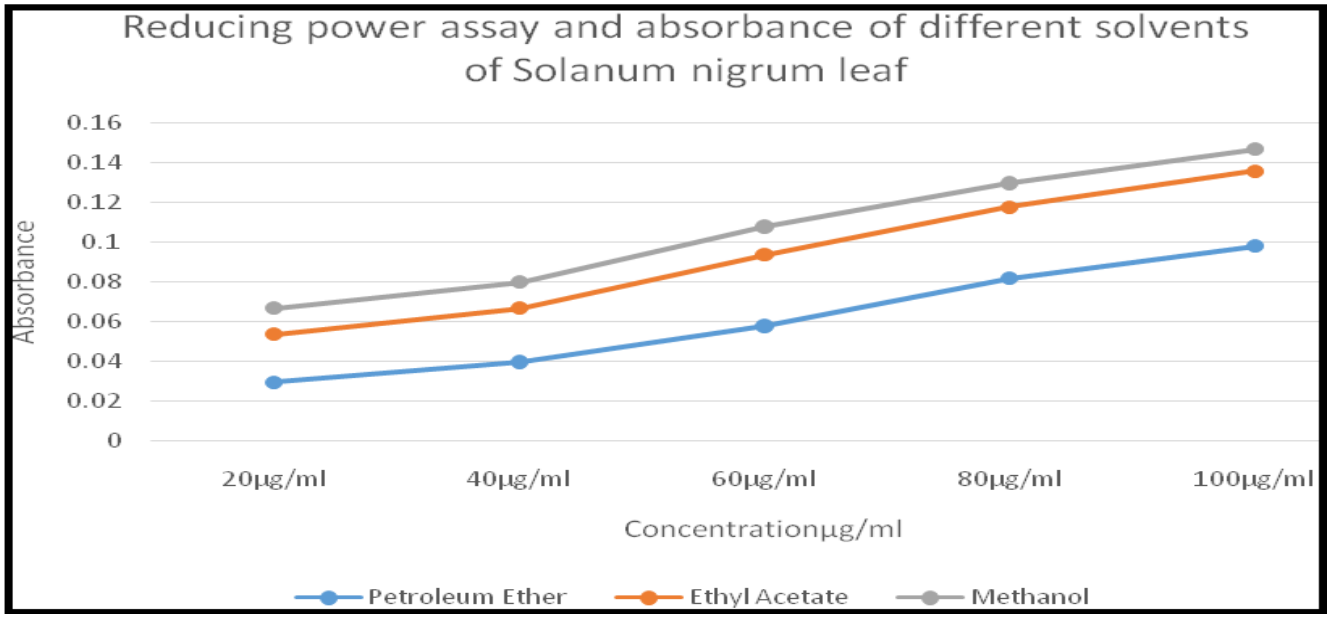
S. No.	Concentration	Absorbance Of Ascorbic acid
1.	10(µg/ml)	0.092
2.	15(µg/ml)	0.111
3.	20(µg/ml)	0.129
4.	25(µg/ml)	0.147
5.	30(µg/ml)	0.159

Table XXIV: Comparison of Reducing Power Assay of different solvent extracts of *Solanum nigrum* leaf

S. No.	Concentration	Absorbance Of Methanolic extract	Absorbance Of Ethyl acetate extract	Absorbance Of Chloroform	Absorbance Of Pet ether
1.	20 µg/ml	0.067	0.054	0.044	0.031
2.	40 µg/ml	0.08	0.067	0.058	0.04
3.	60µg/ml	0.108	0.094	0.084	0.058

4.	80µg/ml	0.13	0.118	0.102	0.082
5.	100µg/ml	0.147	0.136	0.129	0.098





Conclusions

The quantitative analysis of TPC of extracts, indicated that the Methanol extract contains largest amount of TPC (135.16µgGAE/mg), followed by Ethyl acetate extract (68.50µgGAE/mg) and Petroleum Ether (13.00 µg GAE/mg).The quantitative estimation of TFC indicated that methanol extracted it in largest quantity (230.66 µg RE /mg) followed by ethyl acetate (91.33 µg RE /mg) and least by Petroleum Ether (11.00 µg RE /mg). *Solanum nigrum* leaf extracts showed significant antioxidant activities close to that of ascoric acid. Also among all the three leaf extracts used, Methanol extract showed the highest values of inhibition % on the DPPH, depicting that it is a strong antioxidant than Ethyl Acetate and Petroleum ether. Superoxide anions being most toxic species are produced by different biological reactions. Methanolic extract of the plant has the best superoxide scavenging activity, as it shows the lowest values of absorbance and highest values of % inhibition compared to that of the Ethyl Acetate and Petroleum Ether extracts. Methanolic extract may contain

large amount of reductions as compared to Ethyl Acetate and Petroleum Ether extract thus it may function as most efficient electron donors as Ethyl acetate and Petroleum ether extracts and could combine with free radicals to change them into more stable products to check the free radical chain reactions.

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Conflict of interest

The authors declare that they have no competing interest.

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