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In-Vitro Antioxidant Activity of Total Phenolic and Flavonoid Content of *Solanum nigrum* Leaf

Tariq Amir Najar[1], Dr. Suchitra Banerjee[2] and Dr. Rajendra Chauhan[3]
[1]Research Scholar, Barkatullah Vishwavidyalaya Bhopal, Madhya Pradesh, INDIA.
[2]Professor& Head, Department of Biotechnology, I.E.H.E Bhopal, Madhya Pradesh, INDIA.
[3]Professor, Department of Zoology, Govt. M.V.M., Bhopal, Madhya Pradesh, INDIA.

[1]Corresponding Author: tariqamirnajar@gmail.com

ABSTRACT

***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 the extracts of 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 highest antioxidant activity containing highest quantity of phenolics and flavonoids followed by Ethyl Acetate. The presence of phenolic and flavonoid compounds indicated that these compounds can contribute to the 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 system of medicines. The *Solanum nigrum leaf* can be regarded as 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*.

I. INTRODUCTION

The family Solanaceae consists of more than 1500 species and among them many species with cosmopolitant distribution are of economic importance [3]. *Solanum nigrum* belonging to the family Solanaceae, locally known as '*Makoi*' in India 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 proerties. 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 hamper the oxidation of other molecules by inhibiting either the inauguration or extension of oxidizing chain reaction [6]. Antioxidants thus safeguard from the oxidative damage and involve compounds that either eliminate or renew the impaired molecules. They hinder 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[4]. 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[13]. In the present study, the TPC and TFC analysis of the two 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.

II. MATERIALS AND METHODS

2.1. 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 Ethyl Acetate and Methanol for 90 hours.

2.2. 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) [26,3]. 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 FolinCiocalteu reagent and 2ml of a 7.5% sodium carbonate which acts as a buffer to adjust PH value [18]. 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 colour . The colour produced was quantified spectrophotometrically [16,21,14].

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 µg GAE/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 [31]. 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% $AlCl_3$) was added and incubated for 6 minutes, followed by the addition of 2ml of 4% Sodium hydroxide solution (NaOH, 4% w/v) to the mixture and the final volume was made upto 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 were 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.

Y = 0.0014x + 0.1071
R^2 = 0.9437

Antioxidant activity
1.DPPH Radical Scavenging Assay

Methodology:

In DPPH assay, 0.1mM 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 [15]. The free radical scavenging activity was expressed as the percentage inhibition which was calculated by using the following formula:

Inhibition % = (A_0 - A_1 / A_0) x 100

Where A_0= Absorbance of the control,
A_1= 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 colour 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 (%) = $(A_0–A_1) / A_0 \times 100$, where A_0 is absorption of control, A_1 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 (Fe^{3+}) to form potassium ferrocyanides (Fe^{2+}), 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*.,[31]. 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 $FeCl_3$ (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.

III. RESULTS AND DISCUSSION

Total Phenolic Content

Polyphenols like flavonols, phenolic acids etc. are a distinct class of plant phenolic compounds acting as 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 [18]. They are powerful chelators of redox-active metal ions which prevent the transformation of hydroperoxides to reactive oxyradicals by inactivating the chain reactions of free radicals [19]. Significant quantities of phenolics were found in the two 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 exraction [17].

For the phenolic extraction, the efficacy of solvents used was found to be in the order: Methanol > Ethyl acetate. 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 followed by Ethyl acetate (Table III,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). The quantitative analysis of TPC was found to be in the order of Methanolic Extract > Ethyl acetate Extract (ME > EAE).

Table I: 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

Table II: 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 III: 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.

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 [24]. Because of their scavenging ability, flavonoids are known as Reactive Oxygen Species (ROS) Antioxidants delay the process of oxidation by restraining the polymerisation chain reaction begun by free radicals [6]. Phytochemicals being the natural antioxidants have the capacity to relieve the fatal effects of the diseases affiliated to oxidative stress [25]. Phenols and flavonoids are reported to be strong antioxidants because of their hydroxyl groups [9]. 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) [22] 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 [11]. 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) (Table VI,V). The pattern of the quantitative estimation of TFC was found as Methanol extract > Ethyl Acetate (ME > EAE).

Table IV: 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

Table V: 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 VI*:* 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.

Comparison of Total Phenolic and Flavonoid contents of Extracts

The Table (VII) representing the comparison of the Total Phenolic and Total Flavonoid contents in Ethyl Acetate and Methanol Extract. The study revealed that 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 VII: Comparison of Total Phenolic and Total Flavonoid content of 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	Ethyl Acetate	68.50	91.33
02	Methanol	135.16	230.66
	MEAN ± SD	103.69	160.995

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 colourless ethanol solution on reduction in the presence of an antioxidant. This assay helps us to calculate antioxidants by spectrophotometer easily and rapidly [30].

The DPPH assay depends on the potential of an antioxidant to donate an electron or a hydrogen radical to DPPH radical- an inert free radical bearing deep violet colour. 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 [16] and the colour 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 [8].

The Table (viii) 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 (ix) 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 respectiely.

The table (x) 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 Ethyl Acetate and Methanol are inversely proportional to that of the values of their % inhbition and coincides with the study carried out by McCune, stating that the fall in absorbance is directly proportional to the antioxidant concentration [10]. Therefore, the *Solanum nigrum* leaf extracts showed significant antioxidant activities close to that of ascoric acid. Also among the two 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 their efficacy as antioxidants can be expressed as Methanol extract of leaf > Ethyl Acetate extract of leaf (MEL > EAL) (Table xii).

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Table VIII: DPPH Radical scavenging activity of 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 IX: DPPH Radical scavenging activity of Ethyl Acetate extract of *Solanum nigrum* leaf

Ethyl acetate extract of *Solanum nigrum* leaf			
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 X: DPPH Radical scavenging activity of Methanolic extract of *Solanum nigrum* leaf

Methanolic extract of *Solanum nigrum* leaf			
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**	

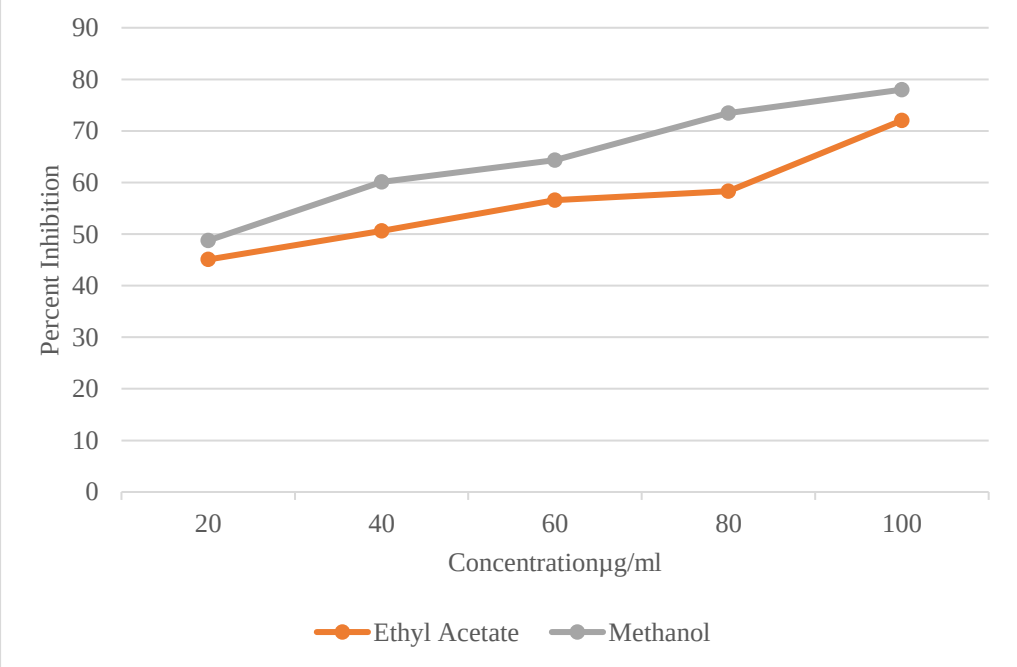

Fig. (a):DPPH Percentage inhibition comparison of two solvent extracts of *Solanum nigrum* leaf

Table XI: Absorbance Comparison of Ethyl Acetate and Methanol extracts of *Solanum nigrum* leaf.

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

Table XII: Comparison of percentage inhibition of Ethyl Acetate and Methanol extracts of *Solanum nigrum* leaf.

S. No	Con. µg/ml	Ethyl Acetate	Methanol
1	20	45.0704	48.7541
2	40	50.5959	60.13
3	60	56.5547	64.3554
4	80	58.2882	73.4561
5	100	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(29). Superoxide anions being most toxic species, are produced by different biological reactions. In the present study the superoxide scavenging activity of the Ethyl Acetate and Methanol Extracts of the *Solanum nigrum* leaf were studied. (Table XIX, XX, XXI). 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 (Table xiii). In Table(Xiv), 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 Xv), 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 the two 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 extracts. The efficiency of these two extracts can be represented in the increasing order as Ethyl Acetate Extract(EAE) < Methanol Extract (ME) (Table xvi).

Table XIII: 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 XIV: Superoxide scavenging activity of Ethyl acetate extract of *Solanum nigrum* leaf

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 XV: 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 XVI: Table representing comparative absorbance of extracts of Ethyl Acetate and Methanol against their concentration.

S. No.	Concentration µg/ml	Ethyl Acetate µg/ml	Methanol µg/ml
1.	20	0..519	0.489
2.	40	0.457	0.444
3.	60	0.419	0.386
4.	80	0.368	0.290
5.	100	0.334	0.237

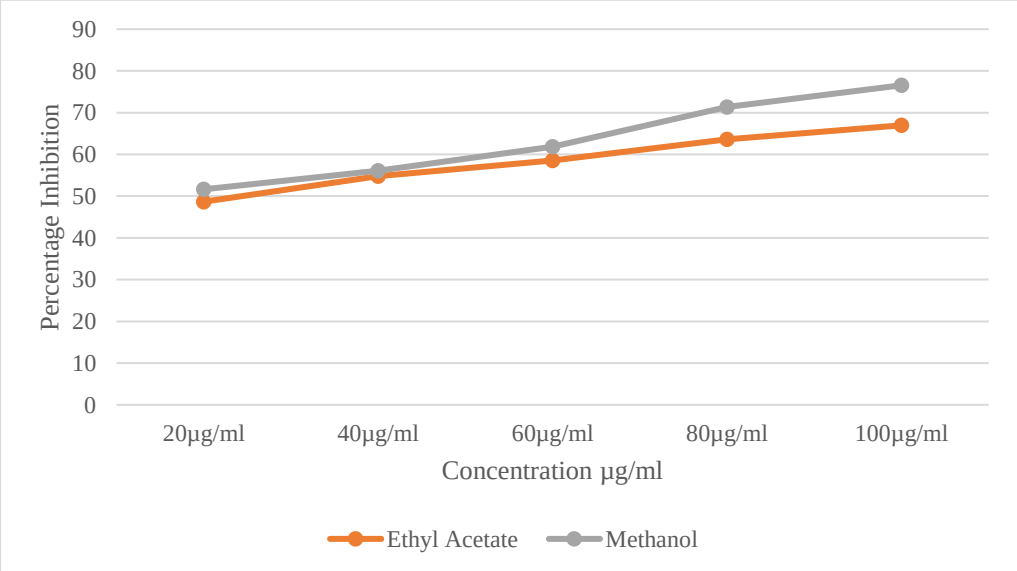

Fig. (b):Superoxide percentage inhibition comparison of different solvent extracts of *Solanum nigrum* leaf

Reducing power assay

Reducing power being related to antioxidant activity may thus act as its important indicator [12]. Compounds with reducing potential signify that they can reduce the oxidized intermediates and act as primary and secondary antioxidants by donating the electrons [26]. The reducing power assay is practised to find out the electron donating ability of an antioxidant [27]. 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 [7]. Although powerful reducing agents produce Perl's Prussian blue colour absored at 700nm. In the present study, Ascorbic Acid has been used as a control (Table XvII) 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(xviii) and Fig.(C) depicting the relative comparison of the increasing conentrations (20, 40, 60, 80, 100µg) of the extracts of Methanolic Extract(ME), Ethyl Acetate Extract (EAE), 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 Extact. In Ethyl Acetate, the absorbance values accelerated from 0.054 < 0.067 < 0.094 < 0.118 < 0.136. Ethyl Acetate Extract showed less degree of Fe^{3+} reduction than 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 the extracts used, Methanolic Extract showed highest values of absorbance and is expressed in the increasing order of asorbance as Ethyl Acetate Extract (EAE) < Methanolic Extract (ME), also reducing power of the extracts used was found to be in the increasing order as Ethyl Acetate Extract (EAE) < Methanolic Extract (ME). The reducing power of a substance is reportedly because of their ability to donate hydrogen (23). Thus, Methanolic extract may contain large amount of reductones as compared to Ethyl Acetate extract thus it may function as most efficient electron donors than Ethyl acetate extracts and could combine with free radicals to change them into more stable products to check the free radical chain reactions.

Table XVII: 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 XVIII: Comparison of Reducing Power Assay of Methanolic and Ethyl acetate solvent extracts of *Solanum nigrum* leaf

S. No.	Concentration	Absorbance Of Methanolic extract	Absorbance Of Ethyl acetate extract
1.	20 µg/ml	0.067	0.054
2.	40 µg/ml	0.08	0.067
3.	60µg/ml	0.108	0.094
4.	80µg/ml	0.13	0.118
5.	100µg/ml	0.147	0.136

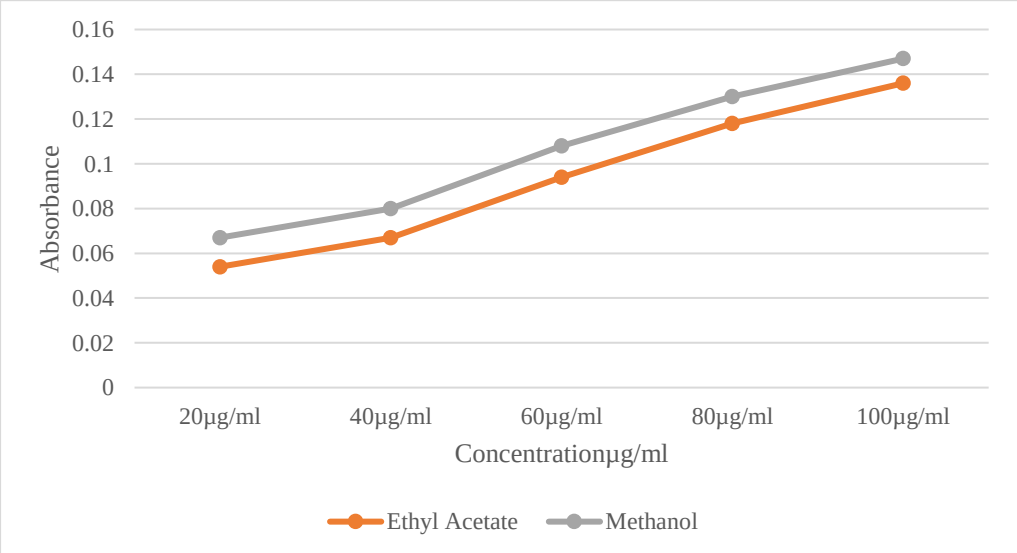

Fig. (c): Reducing power assay and absorbance of two solvents of Solanum nigrum leaf

IV. 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). 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). *Solanum nigrum* leaf extracts showed significant antioxidant activities close to that of ascoric acid. Also among the two leaf extracts used, Methanol extract showed the highest values of inhibition % on the DPPH, depicting that it is a strong antioxidant than Ethyl Acetate. 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. Methanolic extract may contain large amount of reductones as compared to Ethyl Acetate extract thus it may function as most efficient electron donors than Ethyl acetate 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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