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An Assessment of Antioxidant Properties of *Solanum nigrum* and *Tribulus terrestris*

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Abstract:

Tribulus terrestris L. and *Solanum nigrum* L. have been used in traditional practices for centuries. The objective of this study is to analyse the phytoconstituents of hydroalcoholic and ethyl acetate extracts from the fruits of *Tribulus terrestris* L. and *Solanum nigrum*, both qualitatively and quantitatively. Additionally, the antioxidant activity of these extracts will be estimated. Various techniques were used for the analysis of phytochemicals. The antioxidant capability was assessed using several methods including total antioxidant capacity test, hydrogen peroxide scavenging activity, DPPH radical-scavenging activity, nitric oxide radical scavenging activity, and reducing power assay. The qualitative analysis of the fruits of *Tribulus terrestris* L and *Solanum nigrum* L. revealed the presence of many phytoconstituents such as tannins, saponins, flavonoids, and terpenoids. Quantitative analysis showed that the fruits of *Tribulus terrestris* L. included 5.01%w/w flavonoids, 0.70%w/w saponins, and 0.013%w/w terpenoids. On the other hand, the fruits of *Solanum nigrum* L. contained 10.46%w/w flavonoids, 11.02%w/w saponins, and 0.034%w/w terpenoids. Both the extracts of *Tribulus terrestris* L and *Solanum nigrum* L exhibit significant antioxidant activity.

Keywords: *Tribulus terrestris*, *Solanum nigrum*, phytoconstituents, anti-oxidant activity.

INTRODUCTION

Oxidative stress is a significant contributor to a variety of human illnesses. It happens when the production of free radicals suddenly rises owing to a weakened mechanism of protecting antioxidants in our body. The citation "García-Sánchez et al., 2020" is referenced. Reactive oxygen species (ROS) are a group of molecules that include hydrogen peroxide, superoxide, nitric oxide, oxygen-derived free radicals and oxidants, hydroxyl radicals, and reactive nitrogen species (RNS). These molecules are present in the body and are responsible for causing oxidative stress (Phaniendra et al., 2015). These specific types of free radicals possess the capacity to harm DNA, lipids (lipid oxidation), and proteins (protein oxidation) (Brand, 2010; Fransen et al., 2012). Oxidative stress is implicated in the development of various conditions such as hyperglycemia, hypertension, atherosclerosis, diabetes mellitus, cancer,

cardiovascular diseases, Alzheimer's disease, Parkinsonism, and obesity-related syndrome (Fransen et al., 2012; Rajendran et al., 2014; Liguori et al., 2018). Considering the role of oxidative stress in the development of many illnesses, the suppression of oxidative stress might be a promising approach in the treatment of these disorders (Forman & Zhang, 2021).

Solanum nigrum is a member of the Solanaceae family, often known as Black nightshade or Makoi in India (Jain et al., 2011). The aerial portions of this plant are used in traditional medicine to treat snake bites, burns, stomach ulcers, whooping cough, rabies, asthma, and conjunctivitis (Arunachalam et al., 2009; Nitish et al., 2011). The leaves and fruits of *Solanum nigrum* contain a variety of phytoconstituents that have been shown to be useful in treating hepatotoxicity and cytotoxicity (Omara, 2021; Patel et al., 2009). In addition, phytoconstituents such as saponins, flavonoids, and glycosides derived from S. *nigrum* have been discovered to have positive effects as analgesics, anti-inflammatory agents, immunostimulants, anti-diarrheals, anti-cancer agents, antimicrobials, and antioxidants (Rutala et al., 2013; Kalia et al., 2014; Padmashree et al., 2014).

Tribulus terrestris L., a member of the Zygophyllaceae family, is a compact, low-growing shrub that is indigenous to Mediterranean, subtropical, and arid desert regions worldwide, including China, India, Spain, and Bulgaria. It is well recognised as puncture vine or Gokhru (in Hindi) in India and is a highly regarded therapeutic plant in the Indian and Chinese medical systems. T. *terrestris* has been shown to be efficacious in the treatment of several disorders affecting the central nervous system, urogenital system, musculoskeletal system, and gastrointestinal system (Khare, 2007). The aerial plant part of this organism contains various therapeutically active phytoconstituents, including steroidal saponins, glycosides, flavonoids, furostanol saponins, and flavonoid glycosides such as kaempferol and quercetin. These secondary plant metabolites have demonstrated significant pharmacological activity in treating sexual disorders, impotence, and hormonal imbalances.It has been proven effective in treating various illnesses such as cardiovascular diseases, microbial infections, cancer, helminthiasis, renal diseases, and diabetes mellitus.

The present study aims to assess the phytochemical analysis of hydroalcoholic and ethyl acetate extracts obtained from the fruit of S. *nigrum* and T. *terrestris*. This analysis is crucial for future investigations on the anti-obesity properties of these plants. Hence, a phytochemical screening was conducted to assess the antioxidant activity of hydroalcoholic and ethyl acetate extracts derived from the fruit of S. *nigrum* and T. *terrestris*.

Materials and Methodology

Plant Collection, Identification and Preparation

The fruits of T. *Terrestris* and S. *nigrum* were obtained at a local market in Alwar, Rajasthan, India. The verification of both samples was conducted by a Plant Taxonomist from the Department of Botany at Sunrise University in Alwar, India. The plant specimen vouchers may be found in the University herbarium. The obtained, verified fruit and fruits from both plants were rinsed with flowing tap water 3 to 4 times and then dried in a shaded area. Following the drying process, the fruits of T. *Terrestris* and S. *nigrum* were mechanically reduced in size using a grinder. The botanical powder was kept in hermetically sealed containers for future use.

Preparation of extracts

A total of 100 grammes of powdered fruit from the plant T. *Terrestris* was extracted in a sequential manner using a mixture of alcohol and water (60% concentration) and ethyl acetate using a process known as soxhlation, which lasted for a duration of 72 hours. Similarly, a 100-gram powder of fruits from S. *nigrum* was isolated. The extracts were subjected to extraction, filtration, and concentration using a rotary evaporator maintained at 40ºC to generate a semisolid or dry mass. The desiccated raw extracts were used to ascertain the presence and amount of phytoconstituents by qualitative and quantitative analysis, as well as to evaluate their in-vitro antioxidant capacity.

Phytochemical Screening

Phytochemical assessment is a method used to determine the specific chemical components included in a raw plant extract. The techniques used to identify alkaloids, fixed oils, steroids, phenolic compounds, volatile oils, fats, saponins, terpenoids, flavonoids, glycosides, and other substances were selected based on established protocols outlined in the works of Trease and Evans, Harborne, C.K Kokate, and Khandelwal (Harbone, 1987; Nićiforović et al., 2010), as indicated in Table 1: Phytochemical Screening Methods.

Table 1: Phytochemical screening methods

S.NO	PHYTOCONSTITUENT	METHOD	INFERENCE
1	SAPONIN	Few ml of extract + 5ml distilled water, shaken vigorously for 3min.	Formation of foam indicates presence of saponins
2	ALKALOIDS	Few ml of extract + 1ml Dragendorff's reagent +0.2ml acetic acid	Reddish brown precipitates indicate presence of alkaloids
3	TERPENOIDS &STEROIDS	Few ml of chloroform solution of extract + conc. Sulphuric acid, shaken vigorously	Red color on standing indicates terpenoids Lower layer turning yellow indicates steroids
4	GLYCOSIDES	Few ml of Chloroform solution of extract + 0.4ml glacial acetic acid + few drops of ferric chloride +0.5ml conc. Sulphuric acid	Blue color formation indicates presence ofglycosides
5	FLAVONOIDS	Few ml of extract + 1% ferric chloride (alcoholic)	Green color formation indicates presence offlavonoids
6	TANNINS	Few ml of extract + 1% gelatinsolution+10%sodium chloride solution	Precipitate formation indicated presence of tannins
7	CARBOHYDRATES	Few ml of extract + Molish's reagent + conc. Sulphuric acid alongside of test tube	Reddish violet ring indicates presence ofcarbohydrates
8	RESINS	Few ml of extract + water,shaking for few minutes	Formation of turbidity indicates presence ofresins
9	PROTEINS	Few ml of aq. Solution of extract + 10% sodium hydroxide solution, mixed well + 0.1% copper sulphate solution	Formation of violet or pink color indicates presence of proteins
10	AMINO ACIDS	3ml of extract + 3 drops of ninhydrin solution (kept on boiling water bath for10 min)	Purple or bluish color appearance indicates presence of amino acids
11	FIXED OILS	Few drops of extract onfilter paper	Staining of filter paper indicates presence offixed oils

Quantitative estimation of phytoconstituents

Terpenoid content

A solution consisting of methanol and water in a ratio of 4:1 was used to extract the active compounds from 50 grammes of dehydrated powdered plant material. The extraction process was carried out at a temperature of 37º C for a duration of 24 hours. After 24 hours, the mixture was filtered and concentrated at a temperature of 40ºC. 2 million moles of sulfuric acid (H2SO4) were added in a concentrated form and then treated with chloroform (CHCl3). Following the extraction process, the hydro layer was removed and then dried. The remaining substance is referred to as the terpenoidal content, which was determined using the following calculation.

Percentage of terpenoid content=(weight of residue ÷ weight of sample taken)×100

Total Flavonoid Content

10 grammes The plant material was extracted using 100 ml of 80% aqueous methanol at room temperature in triplicates for a duration of 24 hours. The extract was then filtered and subjected to drying on a water bath by putting it into crucibles or China dishes until a consistent weight was achieved. The dried constant mass refers to the amount of flavonoid content found in the plant sample. It was determined using the method described by Liu et al. in 2013.

Percentage of total flavonoid = (weight of residue ÷ weight of sample taken)×100

Total Saponin determination

20 grammes of pulverised plant material was placed in a conical flask and mixed with 100 ml of hydro-ethanol solution (20%). The flask was then placed in a hot water bath and maintained at a temperature below 55ºC for a duration of 4 hours. After a duration of 4 hours, the mixture underwent filtration and the remaining solid material was gathered. The residue was extracted again using 200ml of an aqueous ethanol solution (20%). The extracts were then mixed and reduced to a final volume of 40ml. The 40 ml of concentrated solution was transferred into a separating funnel and subjected to treatment with 20 ml of diethyl ether. The mixture was violently agitated. The layer that developed in the separating funnel is aqueous and was separated and discarded from the other layer (ether). The purified aqueous layer was subjected to additional fractionation using 60ml of n-butanol, which was repeated three times. Subsequently, the fractions of butanol were consolidated and subjected to two treatments with 10ml of aqueous sodium chloride (5%) each. The calculation of the fractions of butanol required to achieve a steady weight % of saponins content was performed according to Sun et al. (2011).

% of Total saponin content=(weight of residue ÷ weight of sample taken)×100

Total Phenolic content (TPC)

The quantification of the total phenol concentration in the extracts was conducted using a slightly modified version of the Folin-Ciocalteu reagent-based spectrophotometric technique. The concentrated solution (1mg/ml) was mixed with 46 ml of water, then 1ml of Folin-Ciocalteu reagent was added and left for 3 minutes. After that, a 2% w/v sodium carbonate solution was introduced. Subsequently, it was maintained for a duration of 180 minutes while being stirred intermittently. The absorbance was quantified at a wavelength of 760nm after the development of a blue colour. The phenolic components were quantified in micrograms of Gallic acid equivalent (GAE) per gramme, as described by Saeed et al. (2012).

In-vitro antioxidant assays

Total antioxidant capacity (TAOC)

A 0.3ml portion of the extract, with concentrations ranging from 50 to 400 µg/ml, was combined with a 3ml combination of reagents consisting of 0.6M sulphuric acid, 28mM sodium phosphate, and 4mM ammonium molybdate. The reaction mixture was incubated at a temperature of 95ºC for a duration of 90 minutes. The combination was cooled to room temperature and then analysed for absorbance at 695nm using a blank as a reference. Ascorbic acid served as the reference substance, and the total antioxidant capacity (TAOC) values were determined in terms of ascorbic acid equivalents (Abu et al., 2017).

DPPH radical-scavenging activity

Various quantities of extract were generated, ranging from 0.1 to 5 µg/ml. 1ml of a 0.1mM ethanol solution of DPPH (2, 2-diphenyl-2picryl-hydrazil) was added to 3ml of extract. The combination of extract and DPPH was left in a dark environment for 30 minutes, after which the absorbance was measured at a wavelength of 517nm. The test was conducted in triplicates, with Ascorbic acid being used as the standard. The outcome was quantified as the percentage of DPPH scavenging effect and calculated using the method provided by Awah and Verla (2010).

"DPPH radical scavenging activity

= {Absorbance(blank) − Absorbance(sample)} ÷ Absorbance(blank) × 100"

Hydrogen peroxide (H_2O_2) scavenging activity

Ascorbic acid served as the reference standard. Various concentrations ranging from 10 to 320 µg/ml were created. One millilitre of each concentration was added to 2.4 millilitres of a phosphate buffer (PBS) with a pH of 7.4. Then, 0.6 millilitres of a 40mM H_2O_2 solution was added. The mixture was vigorously agitated and left at room temperature for 10 minutes. The measurement of absorbance was taken at a wavelength of 230nm. The estimation of hydrogen peroxide scavenging activity was conducted based on the method described by Kitts et al. (2000).

% inhibition=1-(A1-A2)÷Ao×100

Where, Ao= absorbance of control (water instead of sample), A1= absorbance of sample

A2=absorbance of sample in Phosphate Buffer Solution only.

Nitric oxide radical scavenging activity

A solution labelled as AB was newly made by combining 1% sulphonilamide, 2% H_3PO_4, and 0.1% naphthyl ethylenediamine dihydrochloride (Griess reagent). 3 millilitres of extract, with varying concentrations ranging from 10 to 320 micrograms per millilitre, was added to a solution containing 5 millimolar of sodium nitroprusside in phosphate buffer. The mixture was then incubated at a temperature of 25 degrees Celsius for a duration of 2.5 hours. Following this, the solution was treated with newly made solution AB. Ascorbic acid served as the standard. The measurement of absorbance was taken at a wavelength of 546nm. Percent The calculation of inhibition was performed according to Jawad et al. (2013).

% inhibition = (Ao-At)÷A0 × 100

Where, Ao= absorbance of the control (blank without extract) At=absorbance of extract.

Reducing power assay

In this procedure, various quantities ranging from 10 to 320 µg/ml of extracts were produced. 2.5 millilitres of extract was added to an equal amount of sodium phosphate buffer (200 millimolar) and 1% $C_6N_6FeK_3$. The mixture was thoroughly mixed and incubated at a temperature of 50 degrees Celsius for a duration of 20 minutes.

Following this, a small amount (2.5 millilitres) of trichloroacetic acid (10% weight/volume) was added to the mixture, thoroughly mixed again, and then subjected to centrifugation at a speed of 1000 revolutions per minute for a duration of 8 minutes. Following centrifugation, the top layer was isolated. Subsequently, 5ml of deionized water was introduced, along with the addition of 1ml of FeCl3 (0.1%) solution. The absorbance was then measured at a wavelength of 700nm. This method was performed three times and the mean results plus or minus the standard deviation were determined. The EC50 values were determined by analysing the concentration absorbance graph, as reported by Bendary et al. in 2013.

Results and Discussion

Percentage Yield and qualitative phytochemical evaluation

The hydroalcoholic extract of T. *terrestris* had a yield of 2.55%w/w, whereas the ethyl acetate extract had a yield of 0.78%w/w. The hydroalcoholic extract of S. *nigrum* had a percentage yield of 23.77% w/w, whereas the ethyl acetate extract had a percentage yield of 3.32% w/w. Table 2 demonstrates the presence of several phytoconstituents in both plant extracts, as determined via qualitative evaluation.

Table 2: Qualitative analysis of various extracts of *T. terrestris* and *S. nigrum*

S. No	Phytoconstituent	*Tribulus terrestris*		*Solanum nigrum*	
		Hydroalcoholic	Ethyl acetate	Hydroalcoholic	Ethyl acetate
1.	Saponin	+	+	+	+
2.	Alkaloid	-	+	-	-
3.	Terpenoid	+	+	+	-
4.	Steroid	+	+	+	+
5.	Glycosides	-	+	+	+
6.	Flavonoids	+	-	+	+
7.	Tannins	+	+	+	+
8.	Proteins	+	-	+	-
9.	Carbohydrates	-	-	+	+
10.	Amino acids	+	-	+	-
11.	Fixed oils	-	+	+	-

Key: +ve: Present, -ve: Absent

Quantitative estimation of Phytoconstituents

The concentration of flavonoids, saponins, and terpenoids in T. *Terrestris* was determined to be 5.01%w/w, 0.70%w/w, and 0.013%w/w, respectively. The measured concentrations of flavonoids, saponins, and terpenoids in S. *nigrum* were determined to be 10.46%w/w, 11.02%w/w, and 0.034%w/w, respectively. Previous investigations on phytoconstituents have clearly shown their strong antioxidant effects (Côté et al., 2010; Aryal et al., 2019).

Total phenol content (TPC)

These compounds have antioxidant properties due to the presence of -OH groups and phenolic moieties. These phenolic moieties have been shown to stimulate the production of natural antioxidant molecules in the body system (Aliyu et al., 2013). Multiple studies have shown that phenolic compounds have antioxidant action via many

mechanisms, including peroxide breakdown, suppression of free radicals, inactivation of metals, and scavenging of oxygen. These processes effectively prevent oxidative stress. The total phenolic content (TPC) in the hydroalcoholic extract of T. *terrestris* fruits was determined to be 43.6 mg/g GAE (Gallic acid equivalent), whereas the TPC in the ethyl acetate extract of T. *terrestris* fruits was found to be 13.06 mg/g GAE. The total phenolic content (TPC) in the hydroalcoholic extract of S. *nigrum* fruits was found to be 64.01 mg/g GAE, whereas the TPC in the ethyl acetate extract of S. *nigrum* fruits was calculated to be 22.07 mg/g GAE (fig.1). The hydroalcoholic extracts of both plants had a higher total phenol content, indicating a significant antioxidant potential.

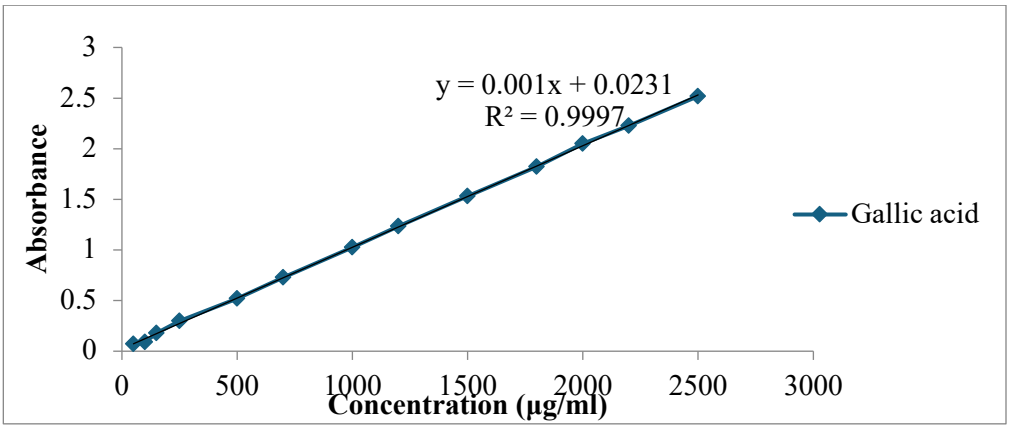

Figure 1: Plot of Gallic acid for estimation of total phenol content

Total antioxidant capacity

This test indicates the presence of antioxidant compounds that are soluble in both water and fat (Kedare & Singh, 2011). The hydroalcoholic extract of T. *terrestris* fruits was found to have a total antioxidant capacity of 36.16µM/g AAE (ascorbic acid equivalent), whereas the ethyl acetate extract of T. *terrestris* fruits had a total antioxidant capacity of 24.7916µM/g AAE. The hydroalcoholic extract of S. *nigrum* fruits exhibited a total antioxidant activity of 42.59 µM/g AAE, whereas the ethyl acetate extract of S. *nigrum* fruits showed an estimated activity of 27.83 µM/g AAE (fig. 2).

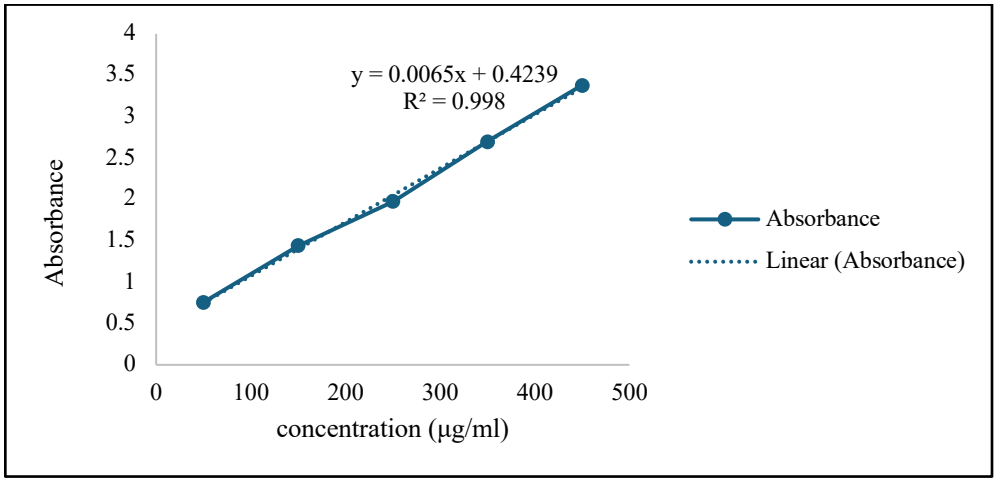

Figure 2: Plot of Ascorbic acid for estimation of Total antioxidant activity

DPPH scavenging activity

The approach is dependable, straightforward, and accurate for evaluating the effectiveness of antioxidants to eliminate free radicals that cause oxidative stress. Additionally, it is excellent for quantifying antioxidants (Razali et al., 2019). The hydroalcoholic extract of T. *terrestris* fruits showed a DPPH scavenging activity of 72.65±0.13% at a concentration of 5µg/ml. The ethyl acetate extract exhibited a scavenging activity of 77.65±0.16%. In comparison, ascorbic acid had a scavenging activity of 84.98±0.11%. The IC50 values for the hydroalcoholic and ethyl acetate extracts of T. *terrestris* fruits were determined to be 2.62 µg/ml and 2.03 µg/ml, respectively. In comparison, the IC50 value for ascorbic acid was found to be 1.58 µg/ml. The hydroalcoholic extract of S. *nigrum* fruits exhibited a DPPH scavenging activity of 72.32 ± 0.11% at a concentration of 5µg/ml. In contrast, the ethyl acetate extract showed a DPPH scavenging activity of 74.65 ± 0.14%. The IC50 values for the hydroalcoholic and ethyl acetate extracts of S. *nigrum* fruits were determined to be 2.40 µg/ml and 2.06 µg/ml, respectively (fig.3). These estimates suggest that both of these plants may be beneficial in managing oxidative stress (Farooq et al., 2019).

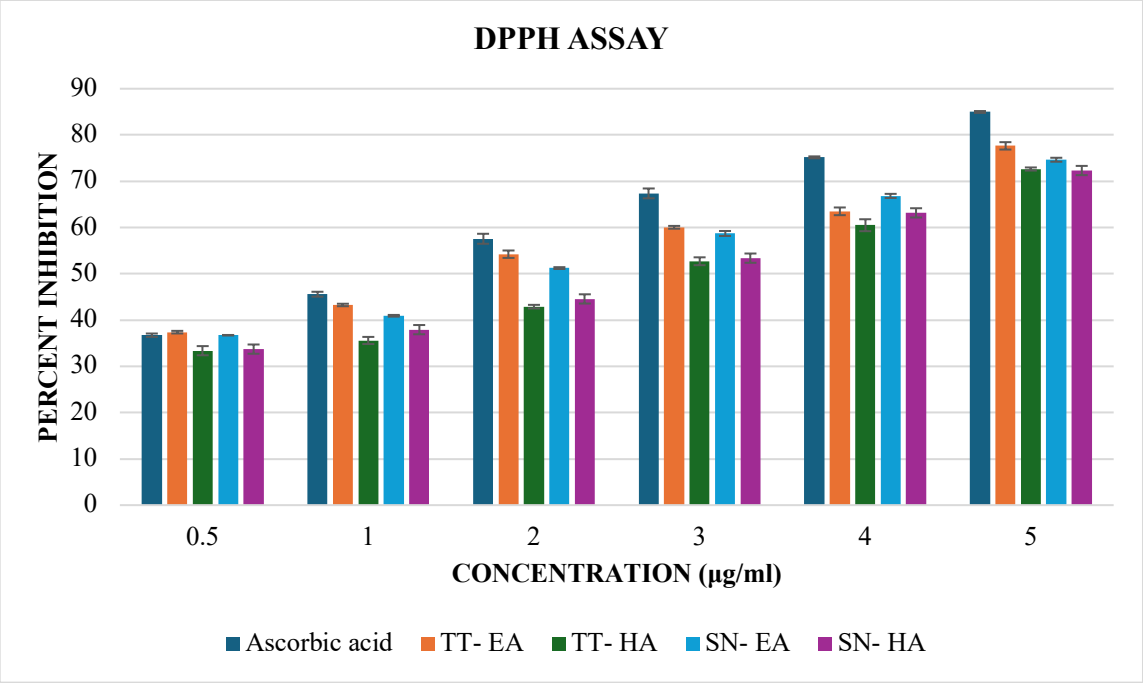

Figure 3: Plot of DPPH scavenging activity of hydroethanolic and ethyl acetate extract of TT and SN.

Hydrogen peroxide scavenging activity

A free radical is a sort of molecule that, when present in excessive quantities, may be harmful to the proper functioning of cells in our body. Therefore, it is important to remove these free radicals by using appropriate antioxidants (Benslama & Harrar, 2016). The hydroalcoholic extract of T. *terrestris* fruits exhibited an antioxidant potential of 74.62±0.61% and the ethyl acetate extract of T. *terrestris* fruits showed an antioxidant potential of 72.55±0.79% against hydrogen peroxide radical at a concentration of 320 µg/ml. In comparison, the antioxidant potential of ascorbic acid was measured to be 89.49±0.19%. The IC50 values for the hydroalcoholic and ethyl acetate extracts of

T. *terrestris* fruits were determined to be 114.94 µg/ml and 121.00 µg/ml, respectively. In comparison, the IC50 value for ascorbic acid was found to be 80 µg/ml. The hydroalcoholic extract of S. *nigrum* fruits exhibited an antioxidant activity of 75.15±0.31% against hydrogen peroxide radicals, whereas the ethyl acetate extract showed an antioxidant activity of 71.65±0.79% at a concentration of 320 µg/ml. The IC50 values of the hydroalcoholic extract and ethyl acetate extract of S. *nigrum* fruits were determined to be 120.93 µg/ml and 126.92 µg/ml, respectively (fig.4). The IC50 values of the T. *terrestris* extracts were lower than those of S. *nigrum*, suggesting that T. *terrestris* has a more effective hydrogen peroxide radical scavenging ability compared to S. *nigrum*.

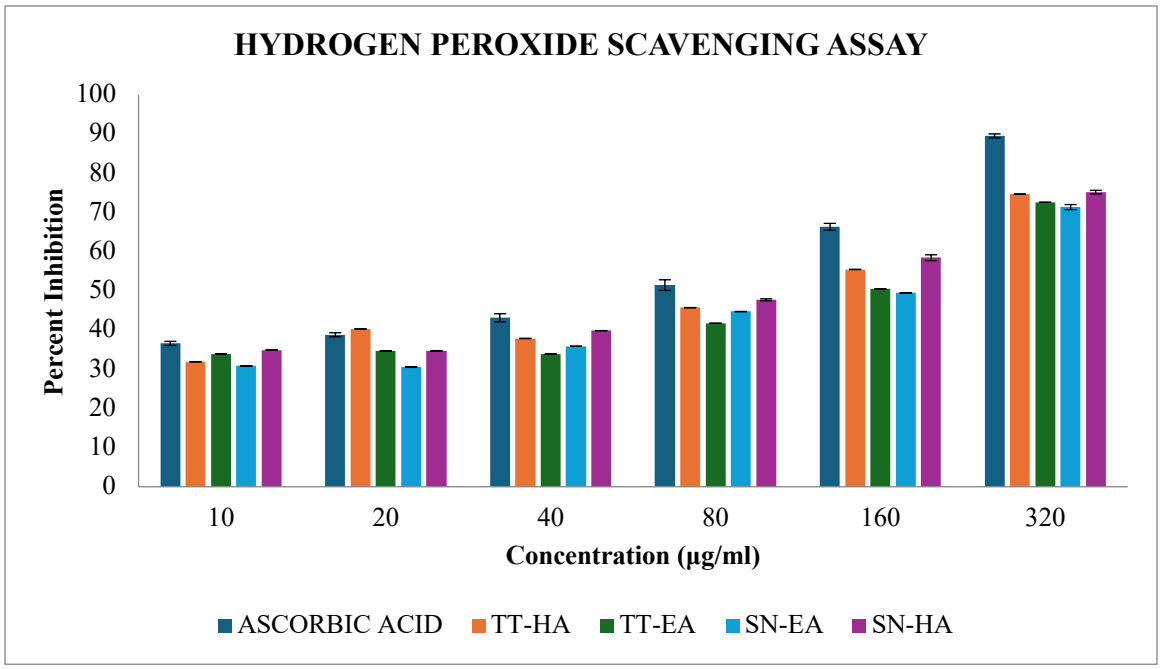

Figure 4: Plot for Hydrogen peroxide scavenging activity of hydroethanolic & ethyl acetate extract of TT and SN

Nitric oxide scavenging activity

This approach entails the decrease in the release of NO (nitric oxide) from sodium nitroprusside due to the struggle between NO and scavengers for oxygen. The hydroalcoholic and ethyl acetate extracts of T. *terrestris* fruits exhibited an anti-oxidant activity of 72.08±0.19% and 71.04±0.22% respectively against the nitric oxide radical at a concentration of 320 µg/ml. In comparison, ascorbic acid had an antioxidant activity of 79.74±0.12% at the same concentration. The IC50 values for the hydroalcoholic extract of T. *terrestris* fruits and the ethyl acetate extract of T. *terrestris* fruits were determined to be 95 µg/ml and 89.13 µg/ml, respectively. The IC50 value for ascorbic acid was determined to be 52.26 µg/ml. The hydroalcoholic extract of S. *nigrum* fruits exhibited an antioxidant activity of 72.06±0.17% and 76.04±0.12% against the nitric oxide radical at a concentration of 320 µg/ml. The IC50 values for the hydroalcoholic and ethyl acetate extracts of S. *nigrum* fruits were determined to be 95.183 µg/ml and 88.80 µg/ml, respectively (fig.5).

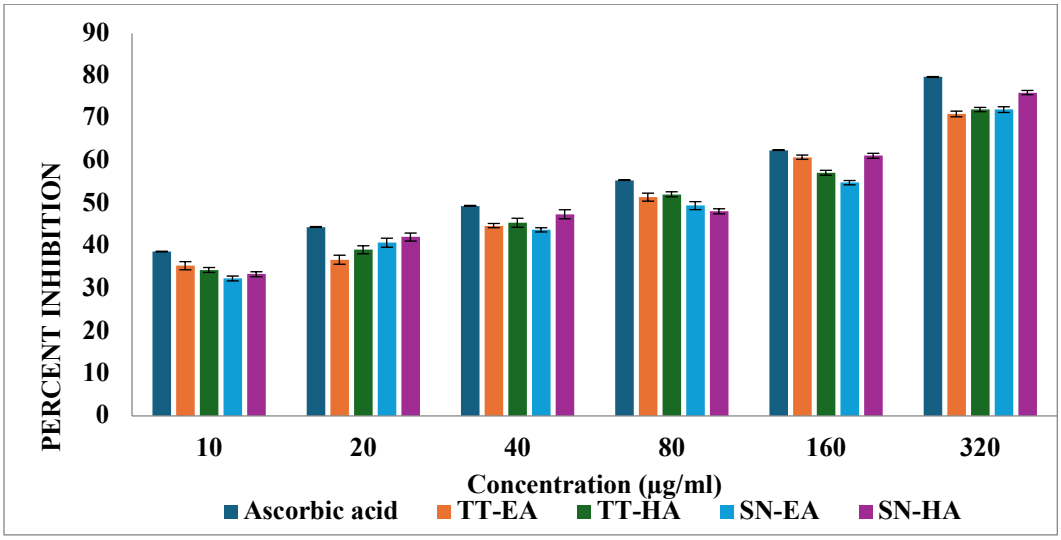

Figure 5.: Plot for Nitric oxide scavenging activity of hydroethanolic & ethyl acetate extract of TT and SN

Reducing power assay

The ability of a molecule to undergo self-reduction is a key characteristic that determines its effectiveness as an antioxidant. The function of these compounds is to decrease the oxidation state of Fe3+ to Fe2+, resulting in the formation of a ferric-ferrous complex when they react with ferric chloride. This may be detected at the absorption peak at a wavelength of 700nm. The EC50 values were determined as 36.16 µg/ml for the hydroalcoholic extract of T. *terrestris* fruits and 24.79 µg/ml for the ethyl acetate extract of T. *terrestris* fruits, whereas ascorbic acid had an estimated EC50 value of 11.70 µg/ml. The EC50 value for the hydroalcoholic extract of S. *nigrum* fruits was determined to be 42.59µg/ml, whereas the ethyl acetate extract of S. *nigrum* fruits had an EC50 value of 27.83 µg/ml (fig.6). The EC50 values of T. *terrestris* fruits were lower than those of S. *nigrum* fruits, indicating that T. *terrestris* fruits have a superior reduction capacity compared to S. *nigrum* fruits.

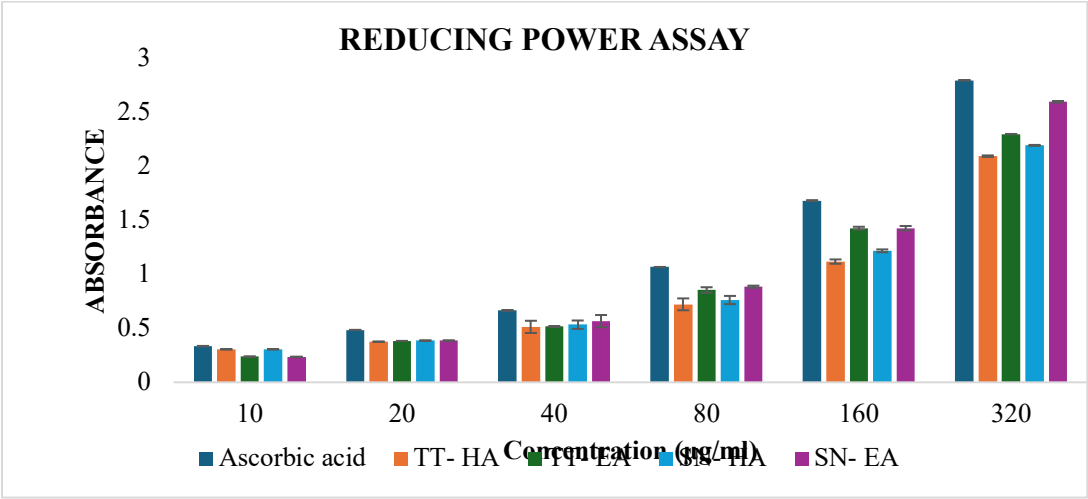

Figure 6: Plot for reducing power activity of hydroalcoholic and ethyl acetate extract of TT and SN

Conclusion

It can be concluded from the results that Solanum nigrum contains a comparable amount of phytoconstituents (flavonoids, saponins and terpenoids) and hydroalcoholic extracts of T. terrestris fruits and S. nigrum fruits have higher antioxidant potential as compared to their ethyl acetate extracts.

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