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Evaluation of Phytochemicals and Validation of Antioxidant Potential of Wild *solanum* species from Mysore District, Karnataka, India

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Abstract

Solanum species are nutritional enriched group of plants recognized for high medicinal value in Angiosperm family. This diverse taxon comprises more than 2,300 species and great deal of studies support the economic importance of these species. However, limited studies have been done on the wild species. The intention of the study was to explore wild species of Solanaceae family for their antioxidant properties. Ten species growing in and around Mysore district, Karnataka, India were screened and phytochemicals from selected species viz *Solanum seaforthianum, S. sisymbriifollium, S. diphyllum* and *S. anguivi* were obtained by soxhlet extraction using methanol, chloroform, and water. Total reducing potential and four radical scavenging assays (DPPH, ABTS, H_2O_2 and NO) were performed and compared with total phenols and flavonoid content. Comparatively, aqueous extract of *S. anguivi* had high phenol (1.55 mg GAE/g) and flavonoid content (96.91 mg QE/g), consequently it displayed maximum reducing potential activity (64.09 mg AAE/g). Although concentration-dependent rise in the radical scavenging efficiency was detected in all species but significant variation ($p<0.05$) was noticed depending on the plant species and extraction solvent used. DPPH and H_2O_2 scavenging was higher in *S. sisymbriifollium* in methanol (97.98%) and chloroform extract (88.85%). *S. diphyllum* displayed more ABTS scavenging efficiency (86.21%) with EC_{50} value of 6.2 mg/ml and *S. anguivi* scavenged NO more efficiently with EC_{50} value of 7 mg/ml. Our findings support the ethno-pharmaceutical properties of selected plants and highlight their possible application in neutralizing free radicals and consequently managing oxidative stress related chronic diseases.

Keywords
Solanum sp., antioxidant, DPPH, ABTS, scavenging, phenols.

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INTRODUCTION:

A physiological condition, where oxidants overwhelm the protective antioxidant system and cause cellular damage is termed “oxidative stress”. In the present scenario, escalation in diseases related to oxidative stress has been a major concern globally. Incomplete processing of oxygen during respiratory chain reaction releases reactive oxygen species (ROS). These are short lived, very unstable, and highly reactive with free electron. Excessive production of ROS acts as mediators and modulates biological macromolecules resulting in consequent

senescence, growth arrest, and apoptosis of cell [1]. If this oxidation process is not controlled, it may lead to oxidative stress causing coronary heart diseases, inflammation, ischemia, carcinogenesis, and age-related disorders [2,3]. Oxidative stress has also been associated with diseases of the central nervous system [4]. Endogenous defensive systems in every organism is equipped with antioxidant molecules that defend and repair oxidative damage. However, in certain cases, these endogenous antioxidants are insufficient and require the exogenous supply of antioxidant.

Antioxidants are also widely applied in food industries to avoid oxidative deterioration of foods. The health hazards and toxicity reported from some of the synthetic preservatives such as butylated hydroxyanisole, butylated hydroxytoluene and propylgallate has increased the need for natural antioxidative agents [5]. In this regard, there is an urge for identifying a potent molecule that can neutralize or reduce the free radicals. Spices, fruits, vegetables, and medicinal plants are some natural sources known to prevent disease related to ROS. In this sense, researchers globally are exploring various plant materials by different assays to evaluate the antioxidant property.

Plant kingdom constitutes diverse groups of chemical compounds and has been used as a source of traditional medicine in several countries. Even in the present era, medicinal plants are gaining incredible popularity because of their reliable and safer bioactive compounds. Hence, phytochemicals are investigated to neutralize free radicals and manage oxidative stress-related diseases.

Solanum spp are ethomedicinal herbs belonging to family Solanaceae and are widely distributed in the tropical and the sub-tropical areas. The group contains highly variable plant species with polymorphic leaf structure, fruit, and stem characteristics. Examples of economically important plants of this family include potato, tomato, and egg plants. Some of the species are cultivated and used as vegetables since they are rich in essential vitamins and minerals [6]. The plant species of Solanaceae produce interesting phytochemicals including steroid alkaloids, polyphenols, hydroxyl cinnamic acid as a self-defense tool against phyto-pathogens [7]. Many of the folkloric reports specify its medicinal value. Fruit of the cultivated *Solanum* sp are generally recommended as dietary supplement for aged, anemic patients and nursing mothers [8]. The bioactive secondary metabolites produced by these plants signify their importance in the field of agriculture, food and pharmaceutical industries. Some of the wild plants species of Solanum are used by tribal and local health practitioners for treating various ailments. Leaf and fruit extract of *S. anguivi* Lam. are applied to reduce itching [9]. Nigerians and Africans consume fruits of *S. anguivi* with a belief of reducing risk of artherosclerosis and diabetes [10]. Roots of *Solanum incanum* L., is used for treating dysentery, asthma, and snake bite [11]. *Solanum nigrum* is used for treating several diseases such as epilepsy and seizure as well as to reduce pain, ulcer, inflammation, infection of eye and jaundice [12,13]. Recent studies have demonstrated their efficiency against liver carcinoma cells, ovarian cancer, colorectal cancer, and endometrial cancer [14,15,16]. *S. torvum* L. is sedative and the fruit decoction is used to reduce cough and treating problems associated with liver and spleen [17]. Some plants contain active molecules such as solanine and solamargine which may cause convulsions and death, when consumed at high dose. *S. sisymbriifolium* has been extensively studied as precursor for contraceptives and liver protection [18]. "Solasodine" isolated from this plant has been demonstrated to be beneficial against ischaemic stroke in rat brains [19].

Despite of enormous effort, metabolic diseases related to oxidative stress such as cancer, cardiovascular problem and neurological disorders remains as a major part of death globally, and warrants for newer and novel molecule that is effective in fighting these diseases. Considering this, the main target of this study was to screen wild species of *Solanum,* for selection of novel plant with antioxidant potential. The phytochemicals were extracted with different solvent systems and analyzed for antioxidant property by five different assays.

MATERIALS AND METHODS:

Reagents, chemicals, and solvents

2,2,-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), peroxidase, synthetic antioxidant standards, tertiary butyl hydroxyl quinine (TBHQ), α-tocopherol (TP), quercetin, ascorbic acid, gallic acid were procured from Sigma-Aldrich, India. Folin-ciocalteau (FC) reagent, sodium carbonate, Aluminium chloride, sodium nitrite, sodium hydroxide, sodium phosphate, sodium chloride, sodium acetate, di potassium hydrogen phosphate, potassium ferricyanide, trichloroacetic acid, ferric chloride, lead acetate, from Sisco Research Laboratory, Bangalore, India. Sodium nitroprusside, Griess reagent, bovin serum albumin (BSA), gelatin, tannic acid, benedict's reagent, copper culphate, dragondroff's reagent, acetic anhydride, ammonia, methanol, acetic acid,

hydrogen peroxide, chloroform, dimethyl sulfoxide (DMSO), sulphuric acid, hydrochloric acid was procured from HiMedia Laboratories, India. All the chemicals and reagents used in the present study are of AR grade.

Collection and processing of plant material

Ten wild species of *Solanum* were collected from different areas of Mysore district, Karnataka, India. The visual morphological identity of plants was confirmed from taxonomic records and local people. Information pertaining to plant size, leaf, flower, and fruit was recorded. The herbarium of these plants was prepared, and the identity was confirmed by taxonomists Dr. K. N. Amruthesh, from Department of Botany, University of Mysore. The voucher specimen has been deposited at Herbarium stock, Department of studies in Botany and the accession number has been obtained. Table 1 represents the details of collected plants with area of collection, identity, and accession number.

Screening of *Solanum* spp. with antioxidant potential

Fresh leaves of selected plants were washed thoroughly in running tap water to remove dirt and spread on blotting paper to air dry. The leaf (10 g) was chopped into small pieces and crushed manually using pestle and motor with 10 ml distilled water. The extract obtained was filtered using muslin cloth and stored in screw cap tubes. The extract was analyzed for total phenol content and DPPH scavenging activity.

Extraction and phytochemical analysis

The washed leaves of selected plants were shade dried and homogenized in a blender to fine powder. The powder was sieved and subjected to extraction of phytochemicals. Soxhlet apparatus was applied for methanol and chloroform extraction. The decoction method with continuous boiling was employed for water extraction. The extracts were then dried under reduced pressure using rotary vacuum evaporator and the percentage yield was calculated. The dried residue was dissolved in DMSO (dimethyl sulfoxide) and stored at -20°C until use. The extract was then qualitatively screened for the presence of phytochemical constituents according to the method described by Harborne [20].

Determination of total phenolic content

The total phenol content in the leaf extracts of selected *Solanum* spp was estimated by FC reagent method as per Gao et al [21]. Briefly, 20 µl of extract (100 mg/ml in DMSO) suspended in 180 µl of distilled water was mixed with 1 ml of 10% FC reagent. The mixture was incubated at 37°C for 5 min. Further, 1 ml of 6% Na_2CO_3 was added and continued the incubation for 30 min. The absorbance was then measured at 750 nm against reagent blank using UV-visible spectrophotometer (CE2021, CECIL, India). The leaf extract replaced with distilled water served as blank. The total phenol content was interpreted by comparing the absorbance reading with standard graph of gallic acid and expressed as amount equivalent to gallic acid (GAE).

Determination of total flavonoid content

Aluminum chloride method elaborated by Kim et al [22] was applied for estimation of total flavonoid content in the extracts. Briefly, 20 µl of extract (100 mg/ml in DMSO) in 4 ml distilled water was mixed with 0.3 ml each of 5% $NaNO_2$ and 10% $AlCl_3$. The mixture was allowed to stay at room temperature for 5 min. Later, 2 ml of 1 mM NaOH was added, and the final reaction volume was made up to 10 ml with distilled water. The absorbance was measured at 510 nm against reagent blank. The blank was prepared by replacing leaf extract with distilled water. The total flavonoid content was calculated by comparing absorbance with standard graph prepared with quercetin and expressed as amount equivalent to quercetin (QE).

Antioxidant activity

Total reducing potential

Total antioxidant capacity or reducing potential of extracts was analyzed according to Oyaizu et al [23]. The reaction mixture containing 20 µl extract (100 mg/ml in DMSO), 180 µl phosphate buffer (0.2M; pH 6.6) and 200 µl of potassium ferricyanide (1%w/v) was incubated at 50°C for 20 min. After incubation, 200 µl of trichloro acetic acid (10% w/v), 0.6 ml distilled water and 94 µl of ferric chloride (0.1% w/v) was added. The precipitate formed was removed by centrifugation (8000 rpm, 10 min) and the absorbance of clear supernatant was recorded at 650 nm. The leaf extract replaced with distilled water served as blank. The reducing potential was calculated by using ascorbic acid standard graph and expressed as amount equivalent to ascorbic acid (AAE).

DPPH scavenging activity

The protocol described by Duan et al [24] was applied for analyzing the DPPH scavenging activity of the extracts. The 2 ml of DPPH solution (0.16 mM in methanol) was mixed with 20 µl of leaf extract (100 mg/ml) and 1.98 ml of methanol. The reaction mixture was incubated in dark at 37°C for 30 min and the intensity of color change was measured at 517 nm against reagent control. Reagent control was prepared by replacing leaf extract with methanol. The percentage DPPH scavenging was calculated with respect to control and the activity was correlated with gallic acid standard.

ABTS scavenging activity

The ability of leaf extracts to scavenge ABTS radical was determined according to Arnao et al [25]. Initially, ABTS reagent was prepared by mixing equal quantity of 7 mM ABTS and 2.4 mM potassium persulfate, followed with 14 h incubation in dark at room temperature. One milli liter of this mixture was then diluted to 60 ml of methanol and used for the assay. The leaf extract was allowed to react with 1 ml of ABTS reagent for 5 min and decrease in color intensity was measured at 734 nm against control. ABTS reagent without leaf extract was used as control. The percentage ABTS scavenging was calculated with respect to control and the activity was compared with tert-butyl-hydroquinone (TBHQ).

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging ability of leaf extracts was determined according to Delpour et al [26]. The 20 µl of leaf extract (100 mg/ml in DMSO) was mixed with 180 µl of 1 mM phosphate buffer (pH 7.4) and 0.6 ml of 40 mM H_2O_2 solution. The mixture was incubated for 10 min at room temperature and absorbance was measured at 230 nm. The reagent control was prepared without leaf extract. The percentage H_2O_2 scavenging was calculated with respect to control and the activity was compared with standard ascorbic acid.

Nitric oxide scavenging activity

The methodology of Panda et al [27] was followed to analyze the nitric oxide (NO) scavenging ability of leaf extracts. Briefly, the reaction mixture containing, 20 µl of leaf extract (100 mg/ml), 1 ml of phosphate buffer (0.2 M; pH 6.4) and 0.5 ml of 10 mM sodium nitroprusside was incubated at 25°C for 60 min. After incubation, equal volume of Griess reagent was added and the absorbance was measured at 540 nm. The leaf extract replaced with buffer served as control. The percentage NO scavenging activity was calculated with respect to control and the activity was compared with standard TBHQ.

Half maximal effective concentration (EC_{50}) determination and correlation with total phenol and flavonoid content

Half maximal effective concentration is the amount of sample required to reduce 50% of free radical. EC_{50} for DPPH, ABTS, H2O2 and NO was estimated by following same protocol with different concentration (2-10 mg/ml) of extracts. The activity was correlated with total phenol and flavonoid content at 95% confidence level and correlation coefficient (R-value) determined.

Statistical analysis

All the experiments were carried out in triplicates and expressed as mean ± standard deviation (SD). The correlation coefficient (R2) for phenol and flavonoid content with antioxidant activity was determined using Graph Pad Prism (Version 8.0.2, GraphPad Software, Inc). A p-value <0.05 was considered significant.

RESULTS:

Screening of *Solanum* spp for antioxidant activity

Fig. 1 displays the external morphological features of ten wild *Solanum* species selected for the present investigation. Each sample showed a distinct shape, with simple to compound leaflets. The edges of leaves vary from regular to irregular, smooth and some with spines. The phenol content in the water extract of these plants varied from 0.26 ± 0.07 to 1.80 ± 0.05 mg GAE/g (Table 2). S. *sisymbriifollium* showed significantly ($p<0.05$) higher phenol content (1.80 ± 0.05 mg GAE/g) followed by *S. nigrum* (1.71 ± 0.01 mg GAE/g). *S. varium* collected from H.D. Kote displayed the lowest phenol content (0.12 ± 0.02 mg GAE/g). The data reveal noticeable ($p<0.05$) variation in the DPPH scavenging activity (Table 2). *S. sisymbriifollium* displayed maximum percentage scavenging (68.81%), followed by *S. anguivi* (38.63%) at a concentration of 1 mg/ml. The percentage scavenging was compared with gallic acid standard and expressed on dry weight basis. Accordingly, higher activity was noticed in *S. sisymbriifollium* (15.26 mg GAE/g) followed by *S. anguivi* (8.56 mg GAE/g). Based on the study, four species namely *S. diphyllum, S. seaforthianum, S. sisymbriifollium* and *S. anguivi* have been selected for further analysis.

Extraction yield and phytochemical analysis

Table 3 represents the extraction yield of methanol, chloroform, and water extracts of leaf from selected *Solanum* species. Methanol extracts comparatively showed higher yield than chloroform and water extract, except in *S. anguivi*, where water used as extraction solvent produced higher yield (31.4%). Among methanol and chloroform extracts, *S. sisymbriifollium* yielded higher percentage of extract (26.0 and 8.13% respectively).

The data on qualitative analysis of twelve different phyto-components reveals the enrichment of methanol extract with tested component compared to chloroform and water extract (Table 3). Phenols, tannins, and flavonoids were present in all the extracts. However, steroids and anthroquinones were completely absent in all the samples. The water extract of all the four plants was devoid of proteins, alkaloids, glycosides, and cardiac glycosides. In chloroform extracts, saponins and terpenoids were not detected.

Total phenols and flavonoids estimation

Fig. 2 displays the total phenols and flavonoids content in the selected plant extracts. The total

phenol content varied from 0.24 ± 0.03 to 1.55 ± 0.03 mg GAE/g, with methanol extract being consistent ($p<0.05$) in the all the four plants. However, significant ($p>0.05$) variation was noticed in chloroform and methanol extracts. The water extract of *S. anguivi* showed maximum phenol content (1.55 ± 0.03 mg GAE/g) which was 3.3 times more than water extract of *S. seaforthianum*. Chloroform extracts displayed lower level, compared to methanol and water extract. Among the four plants species, *S. seaforthianum* and *S. sisymbriifollium* had higher phenol content in methanol extract. However, *S. diphyllum* and *S. anguivi* had maximum in water extract.

Total flavonoid varied from 1.73 ± 0.01 to 96.91 ± 0.13 mg QE/g. In methanol and water extract, maximum flavonoid content was determined in *S. anguivi* (82.00 ± 0.28 and 96.91 ± 0.13 mg QE/g respectively). In chloroform extract, *S. diphyllum* displayed higher flavonoid content (70.91 mf QE/g).

Total antioxidant activity

Reducing potential of phytochemical in each extract was determined by using potassium ferri cyanide. The reduced ferrocyanide reacts with ferric chloride to form green colored complex which is measured at 650 nm. The data show significant variation ($p<0.05$) between the species and their solvent extracts (Table 4). Maximum reducing potential (64.09 mg AAE/g) was observed in water extract of *S. anguivi*, followed by methanol extract (63.81 mg AAE/g). *S. seaforthianum, S. sisymbriifollium* and *S. diphyllum* has better activity in methanol extract and least in chloroform extract.

DPPH Scavenging activity

DPPH radical scavenging activity of extracts showed considerable ($p<0.05$) variation depending on the type of extraction solvent used (Fig. 3a). Among methanol extracts, *S. sisymbriifollium* displayed maximum scavenging (97.98%) followed by *S. diphyllum* (93.46%) at a concentration of 2 mg extract used. *S. seaforthianum* had appreciably higher potential in chloroform extract (78.15%), however among water extracts, *S. sisymbriifollium* showed 94.30% scavenging. Compared to gallic acid standard, most of the extracts possessed marked effect in scavenging DPPH free radical (Table 4). *S. sisymbriifollium* had excellent activity (10.86 ± 0.01 mg GAE/g) in methanol extract. In methanol extract, DPPH scavenging activity decreased in following order S2>S3>S4>S1. In chloroform extract, order of decrease is in the form of S2>S1> S3>S4, however, in water extract the pattern was S2>S4> S1> S3. The EC50 value for the methanol extracts and standard gallic acid is presented in fig. 4. The experimental results show an EC50 value in the range of 6.0 – 7.4 mg/ml whereas the gallic acid showed 11 µg/ml as EC50 concentration.

ABTS scavenging activity

Fig. 3b represents the ABTS radical scavenging capacity of the extracts. The data shows significant ($p<0.05$) variation among the extracts, with methanol extracts showing higher percentage scavenging compared to chloroform and water extracts. *S. diphyllum* had noticeably higher scavenging activity (86.21%) in methanol extract. The activity was compared with standard TBHQ and consequently the maximum activity (5.64 mg TBHQ/g) was evident in methanol extract of *S. diphyllum*, followed by *S. sisymbriifollium* (5.06 mg TBHQ/g) (Table 4). The lowest EC50 value of 6.2 mg/ml was calculated for *S. diphyllum*, whereas the standard TBHQ scavenged 50% of ABTS at a concentration of 7 µg/ml (fig. 4).

Hydrogen peroxide scavenging activity

Fig. 3c shows the effect of solvent extracts from Solanum species in scavenging H_2O_2. The results reveal significantly ($p<0.05$) higher activity in *S. sisymbriifollium* compared to other species. In methanol and chloroform extracts maximum percentage scavenging (68.51% and 88.85%) was observed in *S. sisymbriifollium* and lowest in *S. anguivi* (16.27% and 36.20%). The activity was compared with ascorbic acid standard and determined the highest being 58.00 ± 0.01 mg AAE/g in the chloroform extract of *S. sisymbriifollium* (Table 4). Ascorbic acid standard was calculated to have an EC50 value of 68 µg/ml (Fig. 5). Among the methanol extracts of four selected species, the best EC50 value was determined for *S. sisymbriifollium* (8.5 mg/ml) which was 2 folds lesser than *S. anguivi*.

Nitric oxide scavenging activity

The data reveal a considerable variation ($p<0.05$) in the NO scavenging activity among the species based on the extraction solvent used (Fig. 3d). Comparatively, methanol extracts showed higher activity in all the species. The methanol extract of *S. anguivi* showed maximum percentage scavenging (85.53%), followed by chloroform extract of *S. diphyllum* (71.57%). The activity was compared with TBHQ and the highest activity was calculated for *S. anguivi* (88.54 mg TBHQ/g) (Table 4). The EC50 value for TBHQ was found to be 5 µg/ml and among the methanol extracts, *S. anguivi* was determined to have the best EC50 value (7 mg/ml) (Fig. 5).

Correlation of radical scavenging activity with total phenol and flavonoid content

In the present investigation, the correlation coefficient (R^2) was determined between radical scavenging potential and the total phenols and total flavonoids content using Pearson's correlation

coefficients. Table 5 represents the R^2 value and the p-value for the same. With respect to total phenols, *S. seaforthianum* showed highly significant correlation for H_2O_2 scavenging (R^2=0.99; p=0.0002), whereas *S. sisymbriifollium* and *S. diphyllum* showed high significance with ABTS (R^2=0.99; p=0.0002 and R^2=0.94; p=0.0063). *S. anguivi* showed similar correlation with all the activity (p<0.05). Correlation of activity with flavonoids showed higher correlation in all the samples (R^2>0.95) except ABTS activity in *S. sisymbriifollium* (R^2=0.9; p=0.0144).

Table 1: Details of wild Solanum species collected

Name of the plant	Area of collection	Accession number
S. anguivi	Siddapura, Coorg	UOMBOT22SA61
S. diphyllum	Kukkarahalli lake	UOMBOT22SD62
S. macranthum	Siddapura, Coorge	UOMBOT22SM63
S. nigrum L.	Kukkarahalli lake	UOMBOT22SN64
S. seaforthianum	Kukkarahalli lake	UOMBOT22SS65
S. sisymbriifollium	Thippayana kere	UOMBOT22SS66
S. torvum Sw.	Alanahalli,	UOMBOT22ST67
S. violaceum	Alanahalli,	UOMBOT22SV68
S. xanthocarpum	Thippayanakere,	UOMBOT22SX69
S. varium	Antharasanthe, Kabini forest, H.D. Kote	UOMBOT22SS70

Table 2: Total phenol and DPPH scavenging activity of water extract from selected Solanum species.

Plant name	Total Phenol (mg GAE/g)	Percent DPPH scavenging (%)	DPPH scavenging activity (mg GAE/g)
S. anguivi	1.12 ± 0.04	38.63 ± 0.03	8.56 ± 0.03
S. diphyllum	1.32 ± 0.00	21.02 ± 0.03	4.66 ± 0.03
S. macranthum	0.75 ± 0.07	9.18 ± 0.14	2.03 ± 0.14
S. nigrum	1.71 ± 0.01	4.75 ± 0.03	1.05 ± 0.03
S. seaforthianum	0.62 ± 0.00	17.49 ± 0.02	3.88 ± 0.02
S. sisymbriifollium	1.80 ± 0.05	68.81 ± 001	15.26 ± 0.01
S. torvum	0.58 ± 0.04	17.36 ± 0.02	3.85 ± 0.02
S. violaceum	0.26 ± 0.07	3.91 ± 0.01	0.87 ± 0.01
S. xanthocarpum	1.62 ± 0.01	36.64 ± 0.04	8.12 ± 0.04
S. varium	0.12 ± 0.02	1.94 ± 0.01	0.43 ± 0.01

Table 3: Extractive yield and phytochemical analysis

Parameter	Test	Methanol extract				Chloroform extract				Water extract			
		S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
Extraction yield	**Percentage**	23.9 ± 0.01	26.0 ± 0.00	15.5 ± 0.02	19.7 ± 0.26	7.28 ± 0.11	8.13 ± 0.03	5.10 ± 0.15	3.60 ± 0.21	16.27 ± 0.23	21.2 ± 0.01	8.50 ± 0.11	31.4 ± 0.14
Carbohydrate	Benedict's test	+	+	+	+	+	+	+	+	-	+	-	-
Proteins and aminoacids	Biuret test	+	+	+	+	+	+	+	+	-	-	-	-
Tannins and phenol	$FeCl_3$ test	+	+	+	+	+	+	+	+	+	+	+	+
	Gelatin test	+	+	+	+	+	+	+	+	+	+	+	+
Saponin	Foam test	+	-	+	+	-	-	-	-	+	-	+	+
Flavonoid	Lead acetate test	+	+	+	+	+	+	+	+	+	+	+	+
Terpenoids	Salkowski's test	+	+	+	+	-	-	-	-	+	+	+	+
Alkaloids	Dragondroff's test	+	+	+	+	+	+	-	+	-	-	-	-
Glycosides	Liebermann's test	+	+	+	+	+	+	+	+	-	-	-	-

Cardiac glycosides	Keller kiliani's test	+	+	+	+	+	+	+	+	-	-	-	-
Steroids	Liebermann-burchard's test	-	-	-	-	-	-	-	-	-	-	-	-
Anthraquinone	Borntrager's test	-	-	-	-	-	-	-	-	-	-	-	-

Extractive yield is present as mean ± SD of three independent extractions. S1: *S. seaforthianum*; S2: *S. sisymbriifollium*; S3: *S. diphyllum*; S4: *S. anguivi*. '+' present; '-'not present

Table 4: Antioxidant activity of selected plant extracts

	Methanol extract	Chloroform extract	Water extract
RP	**Activity (mg AAE/g)**		
S1	24.91 ±0.08	0.51 ±0.00	0.53 ±0.01
S2	48.29 ±0.34	0.29 ±0.00	22.94 ±0.03
S3	47.76 ±0.08	1.21 ±0.00	2.07 ±0.00
S4	63.81 ±0.01	0.69 ±0.00	64.09 ±0.08
DPPH	**Activity (mg GAE/g)**		
S1	9.06 ±0.03	8.66 ±0.00	6.02 ±0.03
S2	10.86 ±0.01	7.15 ±0.01	10.45 ±0.01
S3	10.36 ±0.03	3.03 ±0.01	5.21 ±0.01
S4	9.87 ±0.01	2.86 ±0.04	8.99 ±0.01
ABTS	**Activity (mg TBHQ/g)**		
S1	4.07 ±0.03	0.98 ±0.01	0.97 ±0.02
S2	5.06 ±0.02	0.59 ±0.04	4.96 ±0.01
S3	5.64 ±0.00	2.05 ±0.01	2.28 ±0.01
S4	4.15 ±0.01	2.94 ±0.01	2.19 ±0.01
H2O2	**Activity (mg AAE/g)**		
S1	12.33 ±0.00	49.10 ±0.00	3.93 ±0.00
S2	44.72 ±0.01	58.00 ±0.01	45.01 ±0.03
S3	35.37 ±0.01	47.03 ±0.01	6.12 ±0.00
S4	10.62 ±0.00	23.63 ±0.01	5.32 ±0.01
NO	**Activity (mg TBHQ/g)**		
S1	59.12 ±0.00	70.41 ±0.00	51.23 ±0.01
S2	71.20 ±0.00	28.38 ±0.01	54.12 ±0.01
S3	67.52 ±0.00	74.09 ±0.00	54.12 ±0.01
S4	88.54 ±0.00	31.53 ±0.02	52.81 ±0.01

S1: *S. seaforthianum*; S2: *S. sisymbriifollium*; S3: *S. diphyllum*; S4: *S. anguivi*. RP-Reducing potential, AAE-Ascorbic acid equivalent; TBHQ- tertiary butyl hydroxyl quinine

Table 5: Correlation of antioxidant activity with total phenols and flavonoids

Correlation with total phenol								
Assay	**S1**		**S2**		**S3**		**S4**	
	R2	**P value**	**R2**	**P value**	**R2**	**P value**	**R2**	**P value**
DPPH	0.97	0.0027**	0.92	.0091**	0.86	.0229*	0.97	.0028**
ABTS	0.96	.0040**	0.99	.0002***	0.94	.0063**	0.98	.0017**
H2O2	0.99	.0002***	0.89	.0155*	0.86	.0244*	0.98	.0014**
NO	0.93	.0082**	0.95	.0051**	0.90	.014*	0.95	.0050**
Correlation with total flavonoids								
DPPH	0.99	.0007***	0.97	.0018**	0.98	.0010***	0.99	.004***
ABTS	0.95	.0053**	0.90	.0144*	0.96	.0035**	0.98	.0009***
H2O2	0.96	.0034**	1.00	.0001***	1.00	<.0001****	0.96	.0032**
NO	0.99	.0007***	0.98	.0015**	0.99	<.0001****	1.00	<.0001****

S1: *S. seaforthianum*; S2: *S. sisymbriifollium*; S3: *S. diphyllum*; S4: *S. anguivi*.

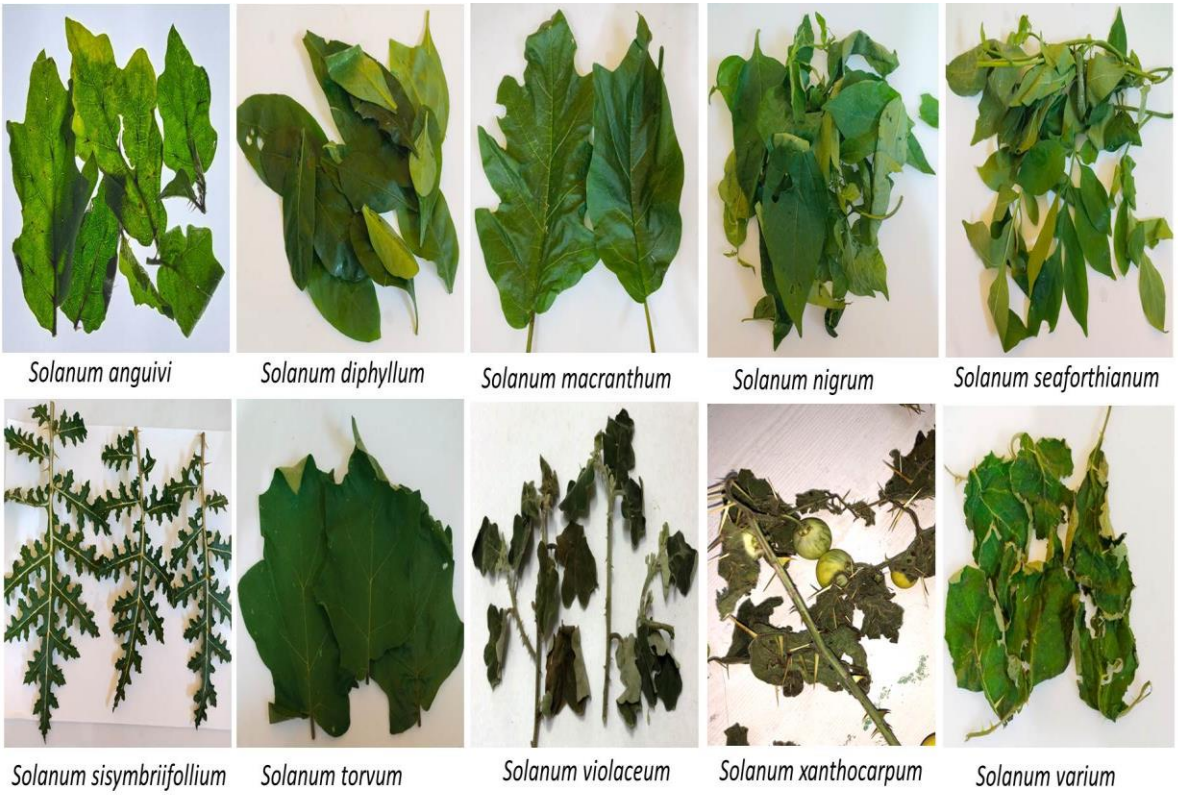


Figure 1: Pictures of leaves from selected wild Solanum species

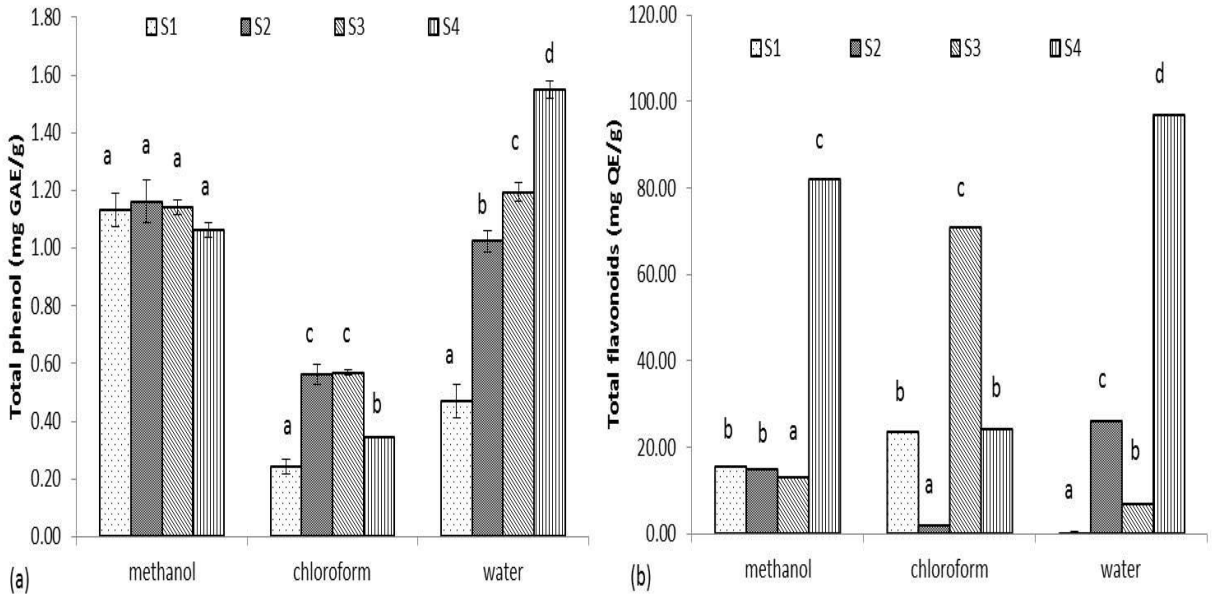


Figure 2: (a) Total phenols and (b) Total flavonoids content in the solvent extract of S1: *S. seaforthianum*; S2: *S. sisymbriifollium*; S3: *S. diphyllum*; S4: *S. anguivi*.

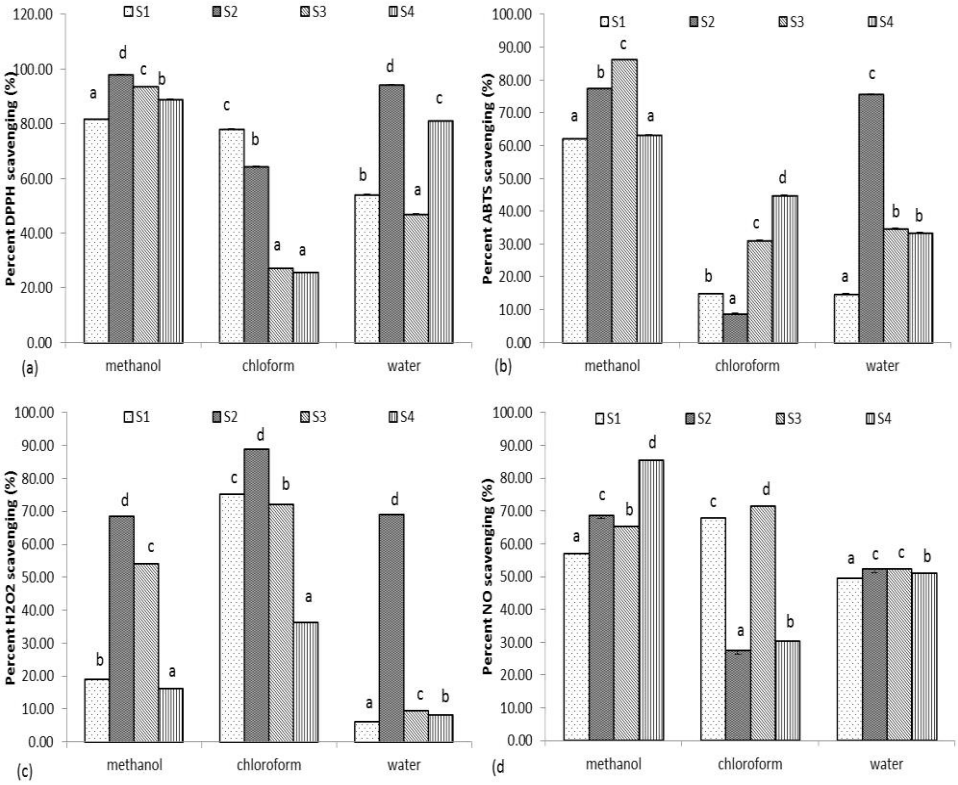
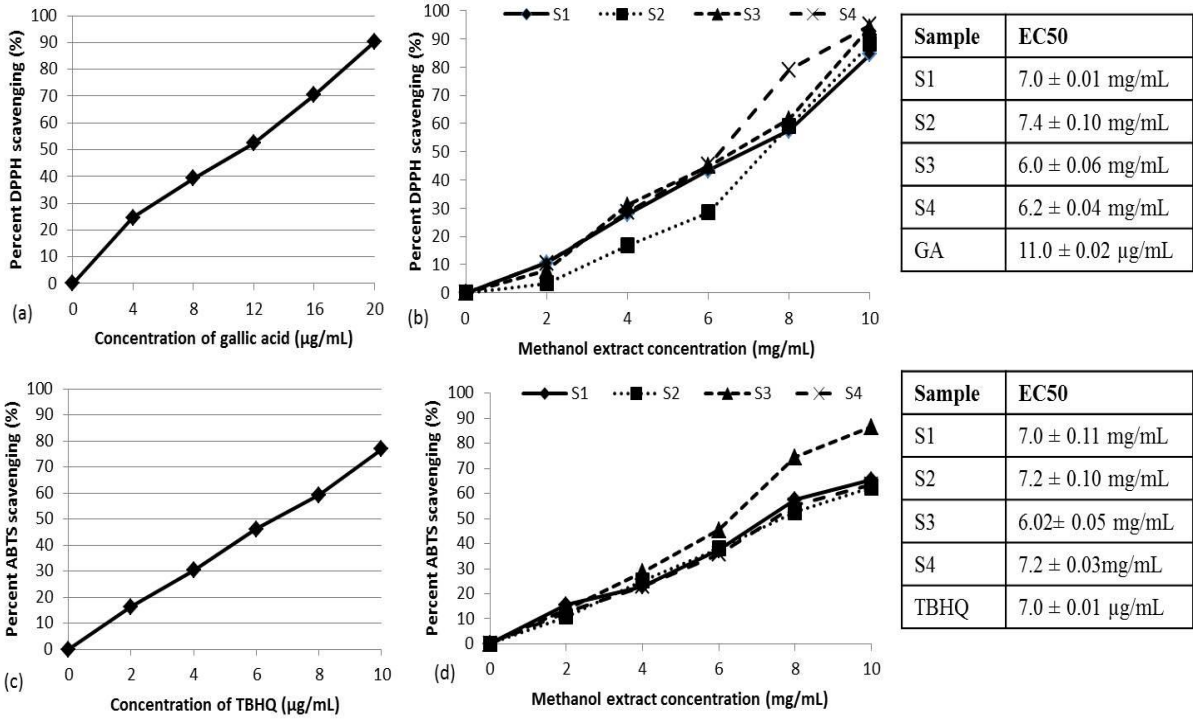


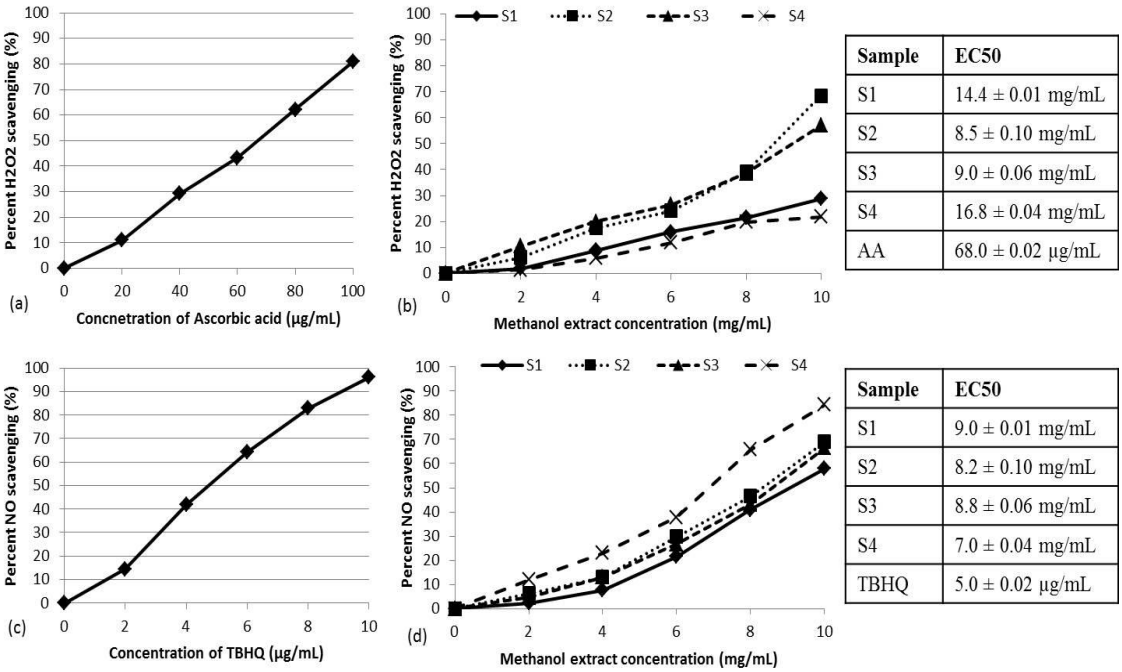
Figure 3: Percentage radical scavenging activity by solvent extracts of selected Solanum species (a) DPPH scavenging activity (b) ABTS scavenging activity (c) H2O2 scavenging activity (d) Nitric oxide scavenging activity. S1: *S. seaforthianum*; S2: *S. sisymbriifollium*; S3: *S. diphyllum*; S4: *S. anguivi*.



Sample	EC50
S1	7.0 ± 0.01 mg/mL
S2	7.4 ± 0.10 mg/mL
S3	6.0 ± 0.06 mg/mL
S4	6.2 ± 0.04 mg/mL
GA	11.0 ± 0.02 µg/mL

Sample	EC50
S1	7.0 ± 0.11 mg/mL
S2	7.2 ± 0.10 mg/mL
S3	6.02± 0.05 mg/mL
S4	7.2 ± 0.03mg/mL
TBHQ	7.0 ± 0.01 µg/mL

Figure 4: Effective minimal concentration (EC50) of the methanol extract of selected Solanum species. (a) Gallic acid standard graph of DPPH scavenging activity (b) DPPH scavenging activity with different concentration of methanol extracts, (c) TBHQ standard graph for ABTS scavenging activity (d) ABTS scavenging activity with different concentration of methanol extracts. S1: *S. seaforthianum*; S2: *S. sisymbriifollium*; S3: *S. diphyllum*; S4: *S. anguivi*.



Sample	EC50
S1	14.4 ± 0.01 mg/mL
S2	8.5 ± 0.10 mg/mL
S3	9.0 ± 0.06 mg/mL
S4	16.8 ± 0.04 mg/mL
AA	68.0 ± 0.02 µg/mL

Sample	EC50
S1	9.0 ± 0.01 mg/mL
S2	8.2 ± 0.10 mg/mL
S3	8.8 ± 0.06 mg/mL
S4	7.0 ± 0.04 mg/mL
TBHQ	5.0 ± 0.02 µg/mL

Figure 5: Effective minimal concentration (EC50) of the methanol extract of selected Solanum species. (a) Ascorbic acid standard graph of H2O2 scavenging activity (b) H2O2 scavenging activity with different concentration of methanol extracts, (c) TBHQ standard graph for nitric oxide scavenging activity (d) Nitric oxide scavenging activity with different concentration of methanol extracts. S1: *S. seaforthianum*; S2: *S. sisymbriifollium*; S3: *S. diphyllum*; S4: *S. anguivi*.

DISCUSSION:

The deleterious effect caused by free radicals raise the concern to scavenge them and inhibit oxidation reaction. There is considerable scientific proof for the potential benefit of antioxidants derived from plant sources to prevent consequences of oxidative stress. In this context, ten wild plants belonging to Solanum genera were screened for their ability to scavenge free radical using in-vitro DPPH assay. Among them, four species namely *S. diphyllum, S. seaforthianum, S. sisymbriifollium* and *S. anguivi* demonstrated the capacity to reduce stable DPPH molecule to diphenyl picryl hydrazine, hence were selected for further studies. In the same line, Gandhiappan and Rengasamy [28] evaluated ethyl acetate extract of five different Solanum species for their antioxidant property and observed intensity of activity in the following order *S. anguivi*> *S. pubscense*> *S. torvum*> *S. trilobatum* > *S. nigrum*> *S. surratense*. Wegener and Jansen [29] compared cultivated (*S. tuberosum* and *S. phureja*) and wild type solanum species (*S. chacoense* and *S. pinnatisectum*) and reported wild species of *S. pinnatisectum* to exhibit higher level of scavenging compared to other selected species.

Solubility of extracted phytochemicals in different experimental models has significant effect on quenching different radicals [30]. Methanol, chloroform and water were used as extraction solvent in this study and compared the antioxidant potential with total phenol and flavonoid content. The classical soxhlet extract method employed was efficient and facilitated easy separation of phytochemicals from complex matrix. Proper extraction technique and suitable solvent system is an essential part for higher extraction yield and biological activity of extracted compound [31]. Polarity of the solvent plays a significant role in the yield and activity of target compound [31]. Variation observed in the extraction yield of present study may be due to the differences in the polarity of the solvent used. Comparatively, methanol fulfilled high efficacy with higher extraction yield.

Kortei et al [32] Compared the phytochemical and antioxidant activity of fruits from three variety of Solanum species, viz., *S. torvum, S. erianthum* and *S. macrocarpon* from Ghana and observed significant affect in the composition based on fruit maturity stage and extraction solvent. Methanol extract of *S. torvum* fruit gave the highest percentage of yield compared to *S. erianthum* and *S. macrocarpon*. Loganayaki et al [33] extracted phytochemicals from *S. nigrum* and *S. torvum* and reported maximum yield in chloroform extract (72.1 and 68.2% respectively).

Acetone and methanol gave lower extractive values. Campisi et al [34] report 26.1% and 19.5% yield with methanol and water extract.

Phytochemicals are secondary plant metabolites that have a functional role in managing several diseases. In the present study, since it was a qualitative method, not much variation was noticed among the species ($p>0.05$) in the respective solvent. However, noticeable discrepancy was seen between the solvent type ($p<0.05$). Ralte et al [35] surveyed ten solanaceae plants of Mizoram, India for its antioxidant and antimicrobial properties. Alkaloids, flavonoids, saponins, tannins and terpenoids were detected in all the plant extracts. Total phenol was highest in *S. anguivi* (29.51 mg GAE/g) and lowest in *S. torvum* (9.87 mg GAE/g). Flavonoid was rich in *S. betaceum* (35.15 mg QE/g) and least in *Capsicum annuum* (8.82 mg QE/g). Total antioxidant capacity was good in *Lycopersicon esculentum* (IC50 34 ug/ml).

Total phenols and flavonoids estimation

Phenolic compounds and flavonoids from plant source have gained intense research interest due to their perceived beneficial effect and safety. These compounds play a key role in scavenging free radicals and transitional metal chelation due to their chemical structure and redox potential [36,37]. Phenols are effective hydrogen donar and hence inhibit lipoxygenase activity [38] Flavonol, flavonones and isoflavones are some of the interrelated compounds of flavonoids that are well studied for curing cancer, inflammation, and cardiovascular diseases [39]. Phenols and flavonoids differed considerably in the selected four plants depending on the solvent used. In fact, flavonoids were higher compared to total phenol content. Phenol content in methanol extract was consistent ($p>0.05$) in all the plants, whereas water extracts showed noticeable variation ($p<0.05$) ranging from 0.47 to 1.55 mg GAE/g. Chloroform extracts showed lower content than water and methanol extracts. *S. anguivi* was enriched with flavonoids, accounting for 96.91 mg QE/g in water extract.

Almoulah et al [40] extracted phytochemicals from Solanaceae species such as *Solanum incanum, S. schimperianum, S. nigrum, Physalis lagascae,* and *Withania somnifera* using methanol and reported total phenol content was in the range of 0.2-0.48 mg GAE/100g. Methanol extract of *S. dulcamara* leaf has been shown to have total phenol content of 21.18 mg GAE/g [41]. *S. nigrum* has been reported to have phenol content of 92.2 and 40 mg GAE/g in methanol and water extract respectively [34]. Total phenolic content was also higher in chloroform extract (5.01 and 8.50 g TAE/100g) of *S. nigrum* and *S. torvum* [33]. Zadra et al [42] evaluated antioxidant activity in solvent extracts of *S. guaraniticum* leaf from Brazil. Total phenol was highest in ethyl acetate extract (546.57 mg GAE/g) with lowest IC50 (9.11 µg/ml) for DPPH. However, chloroform extract showed highest flavonoid content (75.73 mg RE/g)

Hossain et al [43] evaluated phenol content and antioxidant property in different parts of *S. diphyllum* and reported maximum polyphenol content in leaf (68.1 mg GAE/g) and least in roots (26 mg GAE/g). Fruit of *S. anguivi* collected from Nigeria shows 11.13 mg GAE /g total phenols and 9.53 mg QE /g flavonoids [44]. Total phenol content of *S. rostratum* chloroform extract was 88.45 ug GAE/mg (Omar et al 2018). Fruit of *S. anguivi* showed highest phenol and flavonoid content in ethanol extract (202.72 mg GAE/g and 142.64 mg CE/g) as compared to n-hexane and dichloromethane extracts. Correspondently, ethanol extract displayed maximum DPPH scavenging activity (80.13%) with 100 mg/L of sample [45].

Antioxidant activity

Earlier literature validated various assays to measure antioxidant potential of natural extracts [46]. Owing to the type and complexity of oxidant and antioxidant reaction [47], five different assays have been used in the present study with a comprehensive view of assessing the antioxidant activity of extracted samples.

Reducing potential or total antioxidant activity

Reducing potential symbolizes the electron donating ability and serves as substantial indicator of antioxidant capacity. In the assay, the sample extracts were able to reduce ferricyanide complex to ferrous form, thereby confirming the presence of reductants in the extract. The existence of these reductants aid in breaking the reaction chain of free radicals, and thereby protecting against oxidative stress [48]. Among the four Solanum species analyzed, *S. anguivi* had higher reducing potential (64.09 mg AAE/g) related to other species, which had a phenol and flavonoid content of 1.55 mg GAE/g and 96.91 mg QE/g respectively. Loganayaki et al [33] testified methanol extract of *S. nigrum* fruit and chloroform extract of *S. torvum* fruit to display highest total antioxidant activity (15037.4 and 5229.4 uM trolox/g respectively). Reducing power of 64.84% was noticed with 100 mg/L of ethanol extract [45].

DPPH and ABTS radical scavenging activity

DPPH is a nitrogen centered compound commonly used to evaluate radical scavenging efficiency of antioxidant compound. The conversion of dark purple colored DPPH to light yellow colored diphenyl picryl hydrazine in existence of antioxidant agent is

detected through colorometric or spectrometric analysis. The degree of color reduction is directly proportional to the concentration of antioxidant molecule [49]. Similarly, ABTS+ is a protonated free radical, which is dark blue green color in the presence of potassium persulfate. In the presence of hydrogen donating antioxidant, it turns to light green color which can be measured spectrophotometrically [50]. These two radicals were applied in the study to evaluate radical scavenging efficiency of four selected Solanum species. The result show significant variation in the radical scavenging efficiency but showed consistent increment in the activity with increase in dosage. The variation may be attributed to the presence of various compounds like phenols, tannins, saponin, lycopene in the extract [51].

Methanol extract of *S. sisymbriifollium* displayed considerably higher percentage scavenging (97.98%) with EC50 value of 7.4 mg/ml. ABTS quenching capacity was more in methanol extract of *S. diphyllum* (86.21%) with EC50 value of 6.2 mg/ml. Relatively, the extracts showed higher scavenging of DPPH than ABTS. Likewise, Almoulah et al [40] screened methanol leaf extract and steroidal fraction of solanaceae species like *Solanum incanum, S. schimperianum, S. nigrum, Physalis lagascae*, and *Withania somnifera* for their antioxidant property by DPPH and ABTS scavenging assay. The data showed methanol extract to scavenge DPPH better than ABTS. IC50 for DPPH assay was good in *S. schimperianum* (156.1 µg/ml), whereas ABTS scavenging in *S. nigrum* (250 µg/ml). In contrast, Wang et al [52] demonstrated some of plant compounds with higher ABTS scavenging activity than DPPH reduction.

Khalighi-Sigaroodi et al [41] evaluated five species of Solanum (*Datura innoxia, Datura stramonium, S. dulcamara, S incanum* and *S. nigrum*) from Iran and found highest DPPH scavenging activity in methanol extract of *S. dulcamara* leaf with IC50 of 52.51 µg/ml, which had a phenol content of 2118.81 mg GAE / 100g. Leaf extract of *S. diphyllum* displayed the highest DPPH scavenging activity (53.2%) [43]. Gandhiappan and Rengasamy [28] analyzed six solanum species for DPPH scavenging activity and noticed maximum scavenging in ethyl acetate extract of *S. anguivi* and least in *S. surratense*.

Loganayaki et al [33] report *S. torvum* extract to have better DPPH scavenging activity than *S. nigrum*. Among the extracts, acetone fruit extract and chloroform leaf extract showed better activity. ABTS scavenging activity was also maximum in fruit extract (15037.4 µM trolox/g).

Water extract of *S. sisymbriifollium* exhibited highest DPPH scavenging activity with an EC50 value of 131.1 µg/ml compared to ethyl acetate and dichloromethane fraction. Gupta et al [53] analyzed different concentration of *S. sisymbriifollium* methanol extract (40-300 µg/ml) and showed an EC50 value of 211.4 µg/ml. Fruit of *S. anguivi* collected from Nigeria showed IC50 275.03 µg/ml [44]. More and Makola [54] compared water, ethyl acetate and dichloromethane extract of *S. sisymbriifollium* and reported water extract to show higher inhibitory percentage activity (>70%) with an EC50 of 39.4 µg/ml.

Hydrogen peroxide and nitro oxide scavenging ability

Hydrogen peroxide once inside the cells, releases OH radicals that impair cellular function [46]. Hydroxyl ions form 8-hydroxydeoxyguanosine and damage DNA [55]. Hydroxyl radical is another reactive oxygen species that is produced by various biological reactions, especially iron (II)-based fenton reaction. In the same line, NO causes numerous patho-physiological changes in the body through its association with other super oxide radicals. The interaction leads to the formation of dangerous free radicals such as hydroxyl radical or peroxy nitrite anions. Hence the compound which can neutralize these radicals can be an ideal antioxidant agent. Among four Solanum species analyzed, *S. sisymbriifollium* displayed high percentage of H_2O_2 scavenging in all the three extracts. However, *S. anguivi* methanol extract showed higher NO scavenging activity (85.53%) with EC50 value of 7 mg/ml. Gandhiappan and Rengasamy [28] report ethyl acetate extract of *S. anguivi* exhibited strongest hydroxyl radical scavenging activity than other four species tested.

Correlation of antioxidant activity with total phenol and flavonoid content

The present data shows a strong correlation of phenol and flavonoid content with antioxidant activity. Similarly, earlier literature supports the fact that antioxidant activity is mainly attributed by phenolic compounds [28,30,56]. The causative relation between phenol content and antioxidant activity is well-studied and is demonstrated through the hydrogen donating ability of phenols [57,58]. However, the scavenging activity also varies depending on the nature of phenolic compounds, their molecular weight, size, number of aromatic rings, structure of hydroxyl group substitute [59, 60, 61]. In contrast to present study, Almoulah et al [40], the correlation coefficient (R^2) between antioxidant activity and phenolic compounds revealed a value of 0.3727 and 0.1757 for DPPH and ABTS, suggesting no correlation. Loganayaki et al [33] have reported higher ABTS scavenging activity in methanol extract

of *S. nigrum* fruits which has comparatively less phenolic content.

CONCLUSION:

The results of the study suggest the possible role of phenols and flavonoids from selected Solanum spp. in scavenging or neutralizing free radicals to reduce oxidative stress. The wide range of antioxidant activity motivates further identification of lead molecules. Further, analysis is underway to characterize the antioxidant molecule and understanding its nature to implement in functional foods and pharmaceutical preparations. Our future prospective work will be to analyze the efficiency of extracts against enzymes such as catalase glutathione peroxidase and inflammatory cytokines that are involved in progression of oxidative diseases.

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CONFLICT OF INTEREST:

The authors declare that they have no conflicts of interest to disclose.

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