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RESEARCH ARTICLE

Evaluation of Total Phenolic Content, Flavonoid Content and In-vitro Free Radical Scavenging Activity of Solanum indicum Linn.

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ABSTRACT

Objective: To evaluate total phenolic contents, flavonoid contents and *in-vitro* free radical scavenging activity of methanolic extract of leaves of *Solanum indicum* Linn.

Methods: Phytochemical screening, qualitative, quantitative determination of total phenol content (TPC), total flavonoid content (TFC), and *in-vitro* free radical scavenging activity such as DPPH, hydrogen Peroxide (H2O2) and reducing power assay (RPA) were performed using standard procedures.

Results: Phytochemical analysis of methanolic extract revealed the presence of important classes of phytochemicals such as tannins, carbohydrates, alkaloids, terpenoids, glycosides, flavonoids, and others. The total phenolic and flavonoid content of methanolic extract of *S. indicum* leaves was found to be the highest as 101 ± 0.72 and 97.19 ± 2.39 mg/gm of the standard drugs. In *in-vitro* DPPH free radical scavenging analysis, the IC50 methanolic extract was found to be 241.6 µg/mL as compared to standards Ascorbic acid (132.7 µg/mL) and BHT (177.81 µg/mL). In contrast, in hydrogen peroxide scavenging analysis, the IC50 value were found to be 24.30 µg/mL for methanolic extract. In contrast, for standards, it was found to be 21.30 and 23.67 µg/mL for ascorbic acid and BHT, respectively.

Conclusion: According to our findings, the methanolic extract of *S. indicum* leaves is a more powerful antioxidant in comparison with the aqueous extract of *S. indicum* leaves. For this reason present study suggested that leaves of *S. indicum* are a potential source of natural antioxidants. Further study is needed to identify the chemical compounds for various pharmacological attributes.

Keywords: *Solanum indicum*, Antioxidant, DPPH, Hydrogen peroxide, Reducing power assay..

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INTRODUCTION

Oxidation is the chemical process by which an atom, or molecule on robs another of one or more of its electrons. Chemicals exhibiting this tendency to steal electrons are referred to as oxidizing agents. The most familiar oxidizing agent is oxygen itself. Oxidation reactions may involve highly reactive molecules called free radicals. In simple words, free radicals are molecules that have lost an electron and try to replace it by reacting with other molecules. They act as electron acceptors and essentially "steal" electrons from other molecules and free radicals, referred to as oxidizing agents and they tend to cause other molecules to donate their electrons.[1] The most common cellular free radicals are superoxide radical (O_2-*), hydroxyl radical (OH*), and nitric oxide (NO*).[2,5] Other molecules, such as hydrogen peroxide (H_2O_2) and peroxynitrate (ONOO*) are not free radicals but can lead to their generation through various chemical reactions. Free radicals and related molecules are often classified together as reactive oxygen species (ROS) and reactive nitrogen species (RNS) to signify their ability to promote oxidative changes within the cell.[6] The phenomenon of oxidative modification is called as oxidative stress. It is implicated in various diseases like atherosclerosis, Alzheimer's disease (AD), arthritis, Parkinsonism diseases (PD), rheumatism, stroke, and cancer and a long list of degenerative diseases, including aging. Antioxidants are exogenous (natural or synthetic) or endogenous compounds acting in several ways, including removal of O_2, scavenging reactive oxygen/nitrogen species or their precursors, inhibiting ROS formation and binding metal ions needed for catalysis of ROS generation and up-regulation of endogenous antioxidant defenses. The protective efficacy of antioxidants depends on the type of ROS that is generated, the place of generation and the severity of the damage.[7-11]

Solanum indicum Linn. commonly referred to as a Badi. The Sanskrit words Bhatkataiya (Hindi) and `Brihati` are the Ayurvedic medical system can be used as a single drug or as a one drug combination with other medicines. It' could be a much forked perennial below a stinging shrub. The plant grows mainly within the nice and cozy

warm parts 1500 meters above sea level. All plant parts viz. berries, leaves, roots, seeds and stem of this species are utilized in traditional systems of medication and are useful in various diseases like bronchitis, asthma, dry cough, rhinitis, dysuria, leucoderma, sexual disorders, insomnia, cardiac weakness and pruritis.[12-15] The plant has been documented in Chinese folk medicine as an anti-inflammatory and wound-healing agent and as an analgesic for toothache, rhinitis and carcinoma.[16] The species is among the ten medicinal plants whose roots are principally employed in the preparation of Dashmularishta, a well-established Ayurvedic drug utilized in the treatment of fatigue and oral sores. The phytochemical profile of *S. indicum* reported the presence of alkaloids, flavonoids, phenols, and steroids. Clinical studies on antiasthmatic and curing dental inflammation revealed the effective therapeutic potential of *S. indicum*. Exploration of assorted pharmacological effects from *S. indicum* emphasized the possible use of bioactive compounds to develop novel therapeutic drugs. It's a promising approach in the search and development of trendy & stylish medicine, supported by traditional medicinal knowledge.[17]

MATERIALS AND METHODS

Collection and Authentication of Plant Drug

Fresh leaves of *S. indicum* were collected in November 2021 from a locality in Azamgarh, Uttar Pradesh, India, and the specimens (voucher no: NICAIR/ RHMD/ Consult/2020/3600-01) were authenticated by Dr. Sunita Garg (Emeritus Scientist), Raw Materials Herbarium & Museum Department (RHMD) of the National Institute of Science Communication and Information Resources (NISCAIR), PUSA Institute, New Delhi 110012, India.

Chemicals and Standard

Butylated hydroxytoluene (BHT), Ascorbic acid (AA) and I diphenyl-2-picrylhydrazyl (DPPH) were procured from Sigma Aldrich. Sodium carbonate, sodium phosphate, methanol, ethanol, petroleum ether, gallic acid, potassium dihydrogen phosphate, acetic acid (glacial), cupric chloride, concentrated H_2SO_4 (96%) and H_2O_2 (30% v/v) were among the other compounds procured from (Merck KGaA, Darmstadt, Germany). Sodium nitroprusside, ascorbic acid, ferric chloride, naphthyl ethylenediamine dihydrochloride, and folin– ciocalteu's reagent were all provided by Loba Chemie Pvt. Ltd. of India. All of the solvents and reagents that have been utilized were of analytical grade.

Extraction

Leaves of the plant *S. indicum* were air-dried and crushed. About 500 g of the pulverized material was packed in a muslin cloth, separately subjected to a soxhlet extractor, and continuously heat-extracted with (95% w/v) methanol for 72 hours. Then, the methanolic extracts of leaves of *S. indicum* filterate through Whatman Paper No. 42 and the resulting filtrate were concentrated under reduced pressure and finally vacuum dried. The yields of ethanol extract were 11.5% w/w, respectively.

Phytochemical Screening

Standard qualitative tests were used to determine the presence of alkaloids, tannins, saponins, carbohydrates, reducing sugars, proteins, flavonoids, and other phenolic components using simple chemical assays.[12,13]

Determination of Total Phenolic Content

Ainsworth *et al*. described the Folin-Ciocalteu reagent for determining total phenolic content in extracts.[14] One mL of varied concentration plant extracts/standards was combined with 5 mL Folin-Ciocalteu reagent (previously diluted with water l:10 v/v) and 4 mL sodium carbonate (7.5 percent). The tubes were vortexed for a few seconds before being left at 20°C for 30 minutes to develop color. The absorbance of the samples and standard was measured using a spectrophotometer against a blank at 765 nm. The solvent used to dissolve the plant extract was present in a standard blank solution. The following equation was used to calculate the total amount of phenolic content as Gallic acid equivalents (GAE):

$$C= (c\ xV) / m$$

Where; C is the total content of phenolic compounds, mg/gm plant extract, in GAE, c: the Concentration of Gallic acid established from the calibration curve (mg/mL), V is the volume of extract in ml, m is the weight of crude plant extract in gm.

Determination of Total Flavonoid Content

Flavonoid determination was done using the aluminum chloride colorimetric technique.[15] One mL of plant extract/ standard solution was combined with 3 ml of methanol, 0.2 mL of aluminum chloride, 0.2 mL of 1 M potassium acetate, and 5.6 mL of distilled water. After 30 minutes at room temperature, the absorbance of the reaction mixture was measured using a spectrophotometer at 415 nm against a methanol blank. The following equation was used to quantify the total concentration of flavonoid components in plant ethanol extracts as quercetin equivalents:

$$C = (c\ xV/m$$

Where C is the total content of flavonoid compounds in mg/gm of plant extract in quercetin equivalents, c is the concentration of quercetin determined from the calibration curve in mg/ml. of extract, V is the volume

of extracts in ml, and m is the weight of crude plant extract in gm.

Free Radical Scavenging Antioxidant Activity

The DPPH radical scavenging effect of crude methanolic extract of *S. indicum* leaves was calculated using the technique reported by Jain *et al*.,.[16] A solution of 0.1 mM DPPH in methanol was prepared, and 3.0 mL of extract prepared in DPPH methanol containing various concentrations 0.001 to 0.2 mg/mL of the extract was combined with 1.0 mL of this solution (Figure 1). The reaction mixture was vortexed completely and kept at room temperature for 30 minutes in the dark. At 517 nm, the mixture's absorbance was determined spectrophotometrically. The reference standards used were ascorbic acid and BHT. The following formula was used to calculate the ability to scavenge DPPH radicals:

DPPH radical scavenging activity (%) =

$$[(A0-A1)) / (A0)] \times 100$$

Where A0 is the DPPH radical + methanol absorbance and A1 is the DPPH radical + sample extract/standard absorbance.

The 50% inhibitory concentration value (IC_{50}) is defined as the sample's effective concentration necessary to scavenge 50% of the DPPH free radicals present at the outset.

Scavenging of Hydrogen Peroxide

Hydrogen peroxide was used as free radical to test the extract's and sub-fractions' scavenging activities.[17] In a phosphate buffered saline solution, 1-mL of different concentrations of the extract, sub-fractions, and standards in ethanol were added to 2 mL of hydrogen peroxide solution (PBS, pH 7.4). After l0 minutes, the absorbance was measured at 230 nm. The standards were ascorbic acid and butylated hydroxy toluene (BHT). The absorbance of a control sample containing the same amount of extract as the standard was measured using a spectrophotometer at 230 nm.

Reducing Power Assay

The potassium ferricyanide technique was used to test the reducing power.[18] About 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide were combined with one mL of extract and its sub-fractions (final concentration: 5–200 μg/mL). The mixture was then incubated for 20 minutes at 50°C. 2.5 mL of trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 30 minutes. Finally, 2.5 mL of the supernatant solution was collected and mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride, and absorbance at 700 nm was measured. The standard used was ascorbic acid and butylated hydroxytoluene (BHT), respectively, whereas distilled water was used as blank.

Statistical Analysis

The experimental results were expressed as mean ± standard deviation of mean (SD) of three replicates.

RESULT AND DISCUSSION

Phytochemical Screening

Preliminary phytochemical screening of various extracts of leaves of *S. indicum* showed presence of various phytoconstituents ethanolic and aqueous extracts showed the presence of various phytoconstituents methanolic extracts showed the presence of significant phytochemicals like carbohydrate, tannins, alkaloids (ethanol only), terpenoids and flavonoids. The observations were presented in Tables 1 and 2. Studies showed that leaves of *S. indicum* possess a plethora of phytochemicals.[19]

Total Phenol Contents

Using the Folin-Ciocalteu reagent, the total phenolic content of *S. indicum* extract was measured and represented as gallic acid equivalents (GAE)/gm of plant extract. Using the gallic acid standard curve (y = 0.0087x-0.004; R2 = 0.9993), the total phenolic content of the test fractions was determined (Figures 2 and 3). The highest level of phenolic content was present in the methanolic extract of *S. indicum* leaves. The extracts' phenolic contents decreased in the following order: methanolic extract (101.11 ±.072 μg/mL) (Table 3). Phenolic chemicals, in general, can trap and neutralize free radicals, protecting our cells against the aging process and various diseases. Moreover, high phenolic content in plants is associated with anticancer and disease-prevention abilities.[20] The methanolic extracts of *S. indicum* leaves had higher DPPH radical scavenging activities indicated with IC_{50} value of 241.6 μg/mL, according to this study. The presence of several phenolic compounds and other phytochemicals, which are a rich source of antioxidants, was shown to be responsible for the therapeutic capabilities of the *S. indicum* leaves (Table 4).

Total Flavanoid Contents

The total flavonoid content of *S. indicum* leaves is shown in Table 5. The total flavonoid content was calculated using a standard curve (y = 0.006x-0.079; R2 = 0.996) and expressed as mg of quercetin equivalent/g of extract. (Figure 4) The highest quantity of flavonoid content was detected in the methanolic extract of *S. indicum* leaves. The flavonoid concentration of the extracts was

Table 1: Phytochemical screening of *S. indicum*

S.No.	Constituents	S. indicum
1	Alkaloids	+
2	Carbohydrates	+
3	Glycosides (Cardiac and Anthraquinone glycosides)	-
4	Phenolic compounds and tannins	+
5	Flavonoids	+
6	Terpenoids	+
7	Saponins	+
8	Sterols	+
9	Proteins	+
10	Resins	-

+ = Present, - = Absent

Table 2: Preliminary phytochemical investigation of different extracts of *Moringa oleifera* Lam leaves

Phytochemicals	Tests	I	II	III	IV
Carbohydrate	Molisch's	-	-	+	+
	Fehling's	-	-	+	+
	Barfoed test	-	-	+	-
Tannin	Ferric chloride	-	-	+	+
	Gelatin	-	-	+	+
Alkaloid	Dragendorff's	-	+	+	+
	Hager's	-	+	+	-
	Mayer's	-	+	+	-
	Wagners	-	+	+	-
Terpenoids	Salkowski	-	-	+	+
Steroids	liebermann	+	+	-	-
Glycoside	Kellar killani	+	+	+	-
Saponin	Foam test	-	-	-	-
Flavonoid	Shinoda	-	-	+	+
	Lead acetate	-	-	+	+

I-Pet. Ether extracts, II-Chloroform Extracts, III-Ethanolic Extracts, IV- Aqueous Extract - Abscent, + Present

Table 3: Content of total phenolic in methanolic extract of *S. indicum*

S. No	Absorbance	Intercept	Slope	Conc. (µg/mL)	Mean
1.	0.8032	0.004	0.008	101.70	101.11 ±.72
2.	0.7921	0.004	0.008	99.67	
3.	0.8069	0.004	0.008	101.97	

shown to decrease in this order: Methanolic extract (97.19 ± 2.39 mg/gm) (Table 5). The methanol soluble component of the plant contained more flavonoids than the aqueous soluble fraction, according to the overall flavonoid content. Depending on the phenolic content of extracts, solvent extraction has been shown to play

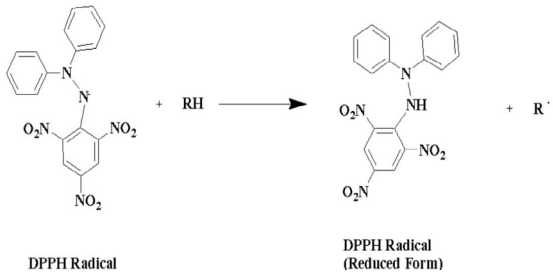

Figure 1: DPPH free radical scavenging

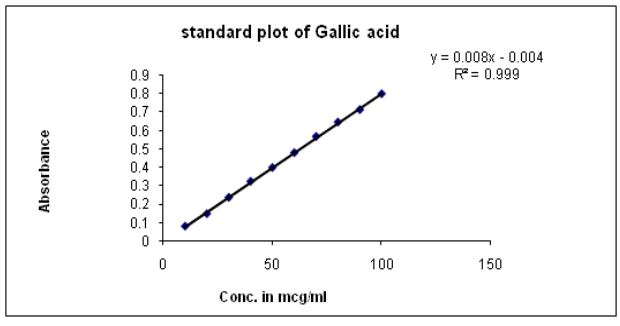

Figure 2: Calibration curve of gallic acid for total phenolic content

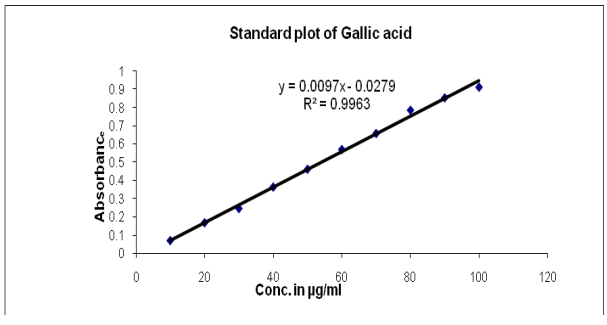

Figure 3: Calibration curve of gallic acid for total phenolic content

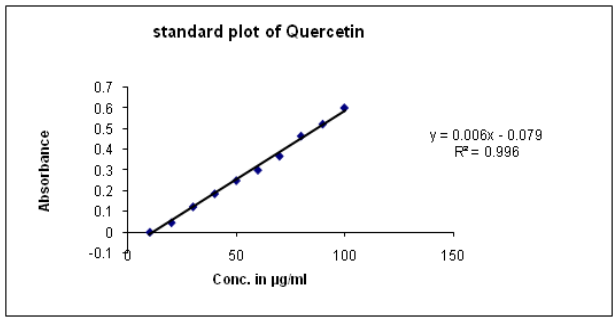

Figure 4: Calibration curve of quercetin for total flavonoid content

a significant role in their antioxidant potential.[21] The presence of hydroxyl (OH) groups in phenolic compounds may contribute directly to their antioxidant activity and be a significant predictor of their radical scavenging ability (Table 6).[22]

DPPH Free Radical Scavenging

The DPPH radical scavenging activity of *S. indicum* leaves extracts revealed a significant and concentration-

Table 4: Total phenolic content of different extract of *M. olifera* Lam. leaves

MO Leaves extract	*Total phenolic contents (mg/gm, Equivalent to Gallic Acid)*
Ethanolic Extracts	99.24 ± 0.42
Aqueous Extracts	43.13 ±0.062
Chloroform Extracts	21.80 ± 0.98
Petroleum Ether Extracts	11.05±0.60

Data represent Mean ± Standard deviation of triplicate analysis

Table 5: Content of total flavonoids in methanolic extract of *S. indicum*

S. No	*Absorbance*	*Intercept*	*Slope*	*Conc. (µg/mL)*	*Mean*
1.	0.5126	0.079	0.006	92.5	97.19 ± 2.39
2.	0.5341	0.079	0.006	100.37	
3.	0.5213	0.079	0.006	98.71	

Table 6: Total flavanoid contents of different extract of *M. olifera* Lam. leaves

MO leaves extract	*Total flavanoid contents (mg/gm, Equivalent to Quercetin)*
Ethanolic extracts	91.72 ± 1.98
Aqueous extracts	45.76 ± 1.85
Chloroform extracts	10.40 ± 1.30
Petroleum ether extracts	3.36 ± 0.69

Data represent Mean ± Standard deviation of triplicate analysis

dependent increase in scavenging impact, with the methanolic fraction of the leaves being the most active. The capacity of various extracts to diminish DPPH, a stable free radical, was investigated. Any molecule that can contribute an electron or hydrogen to DPPH can react with it and therefore, bleach the DPPH absorption. DPPH is a purple-colored dye with a maximum absorption wavelength of 517 nm. When it reacts with a hydrogen donor, it converts to 2, 2-diphenyl-lpicryl hydrazine, which results in a lowering in absorbance.[23] We chose ethanol and aqueous extract for antioxidant properties among on the basis of quantitative analyses. At 1000 g/mL, the methanol extract had the highest activity of 74.80%, compared to ascorbic acid and BHT had 83.69 and 76.86% inhibition, respectively. The IC_{50} values of the methanolic extracts suggested that they had significant DPPH free radical scavenging activity, as shown in (Table 7 and Figures 5 and 6). The IC_{50} value represents the scavenging activity's potency. The IC_{50} of standard ascorbic acid and BHT were determined to be 132.7 and 1.77.8 µg/mL, respectively. The IC_{50} of methanol extract of *S. indicum* leaves was 241.6 µg/mL (Table 8).

Scavenging of Hydrogen Peroxide

Despite the fact that hydrogen peroxide would not be

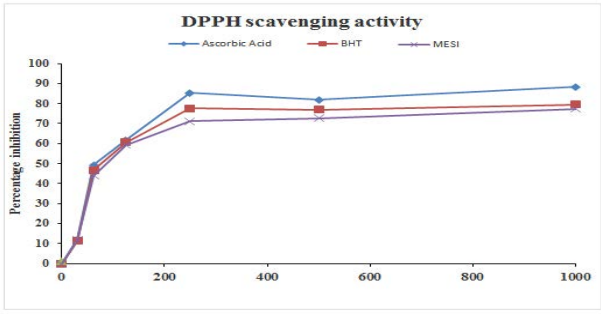

Figure 5: Comparative DPPH radical scavenging activity of MEAP, ascorbic acid and butylated hydroxy toluene (BHT)

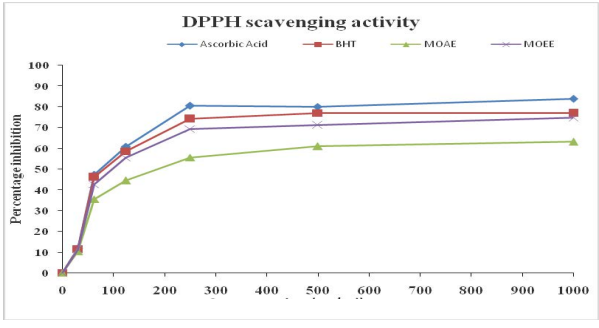

Figure 6: Comparative DPPH radical scavenging activity of MOEE, MOAE, ascorbic acid and butylated hydroxy toluene (BHT)

a radical, it contributes to oxidative stress. Even small amounts of H_2O_2 may be significant in biological systems. Inside the cell, naturally, exists iron complexes are likely to react with H_2O_2 *in-vivo* to produce extremely reactive hydroxyl radicals, which might be the source of many of the cell's harmful effects.[24] Figure 7 shows the scavenging of hydrogen peroxide by various preparations of *S. indicum* leaves (Table 9 and Figure 8). The methanol extract showed excellent activity in depleting H_2O_2, with IC_{50} values of 24.30 µg/mL (Table 10).

Reducing Power

The reducing power was measured by using ferric-to-ferrous reducing activity, which was quantified spectrophotometrically from the production of Perl's Prussian blue color complex.[25] Ascorbic acid and BHT were used to examine the reducing power of various *M. oleifera* leaves extracts as standards (Figure 9). The methanolic extract of *S. indicum* has the highest reducing power. This finding suggests that the methanol extract of *S. indicum* leaves may contain polyphenolic chemicals with strong reducing properties. The fact that methanol extract is the most reducing agent with the maximum phenolic content has been used to justify the claim (Figure 10). The most effective reducing agents were ascorbic acid, BHA, and BHT used as standards. The concept behind this approach is that the absorbance of the reaction mixture increases. An elevation in absorbance suggests

Table 7: Percentage inhibition of free radical scavenging activity by test and standard drugs and their IC_{50}

Drugs	*0*	*31.25*	*62.5*	*125*	*250*	*500*	*1000*	*IC_{50}*
Ascorbic Acid	0	11.92	49.17	61.79	85.25	81.97	88.32	132.70
BHT	0	11.34	46.71	60.65	77.49	76.95	79.51	177..8
MEAP	0	11.67	43.78	59.43	71.24	72.65	77.29	241.6

Table 8: Percentage inhibition of free radical scavenging activity by test and standard drugs and their IC_{50}

DPPH % Scavenging Activity											
Conc. (µg/mL)	*%Inhibition*	*IC_{50} value*	*Conc. (µg/mL)*	*%Inhibition*	*IC_{50} value*	*Conc. (µg/mL)*	*%Inhibition*	*IC_{50} value*	*Conc. (µg/mL)*	*%Inhibition*	*IC_{50} value*
Ascorbic Acid			*BHT*			*MOAE*			*MOEE*		
31.25	11.72		31.25	11.52		31.25	10.35		31.25	11.23	
62.5	47.17		62.5	46.09		62.5	35.45		62.5	42.58	
125	60.55	122.7 µg/mL	125	58.50	167.8 µg/mL	125	44.43	456.9 µg/mL	125	55.57	237.6 µg/mL
250	80.37		250	74.12		250	55.47		250	69.34	
500	79.98		500	76.95		500	60.84		500	71.09	
1000	83.69		1000	76.86		1000	63.09		1000	74.80	

MOAE-Moringa oleifera Aqueous Extract, MOEE-Moringa oleifera Ethanolic Extracts

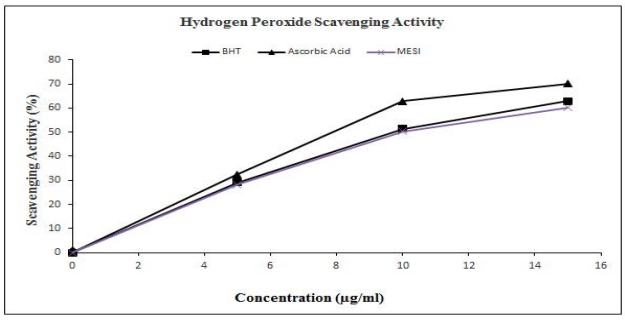

Figure 7: Comparative hydrogen peroxide scavenging activity of MEAP, ascorbic acid and butylated hydroxy toluene (BHT).

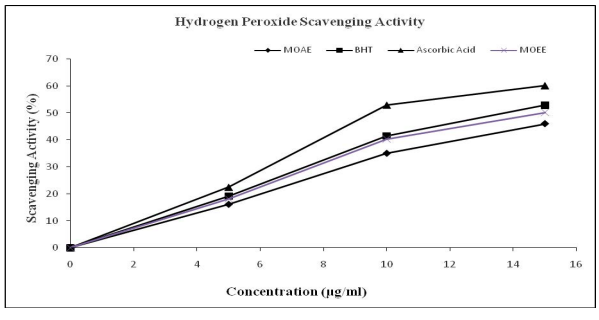

Figure 8: Comparative hydrogen peroxide scavenging activity of MOEE, MOAE, Ascorbic acid and Butylated hydroxy toluene (BHT)

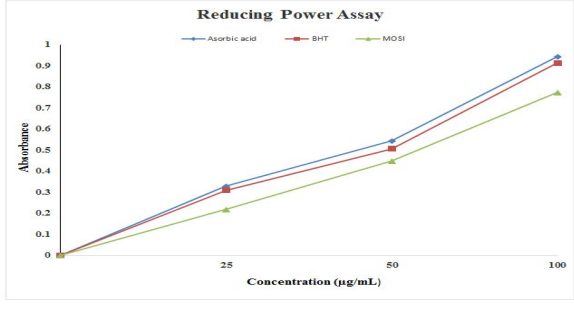

Figure 9: Comparative reducing power scavenging activity of MEAP, ascorbic acid and butylated hydroxy toluene (BHT).

Table 9: Percentage inhibition of hydrogen peroxide scavenging activity by test and standard drugs and their IC50

Conc	*0*	*5*	*10*	*15*	*IC_{50}*
BHT	0	29.18	51.45	62.88	23.67
Ascorbic Acid	0	32.44	62.88	70.1	21.3
MEAP	0	28.16	50.33	60.16	24.3

that antioxidant activity is increasing. The reducing power of the samples is shown by an increase in the absorbance of the reaction mixture.[26] Reducing power has been attributed to antioxidant activity and may be

Table 10: Percentage inhibition of hydrogen peroxide scavenging activity by test and standard drugs and their IC50

Hydrogen peroxide %scavenging activity											
Conc. (µg/mL)	*%inhibition*	*IC_{50} value*	*Conc. (µg/mL)*	*%inhibition*	*IC_{50} value*	*Conc. (µg/mL)*	*%inhibition*	*IC_{50} value*	*Conc. (µg/mL)*	*%inhibition*	*IC_{50} value*
Ascorbic Acid			*BHT*			*MOAE*			*MOEE*		
5	22.44		5	19.18		5	16.13		5	18.16	
10	43.75	11.30 µg/ml	10	41.45	13.67 µg/ml	10	34.99	15.92 (µg/ml)	10	40.33	14.30 (µg/ml)
15	60.10		15	52.88		15	45.94		15	50.16	

MOAE-Moringa oleifera Aqueous Extract, MOEE-Moringa oleifera Ethanolic Extracts

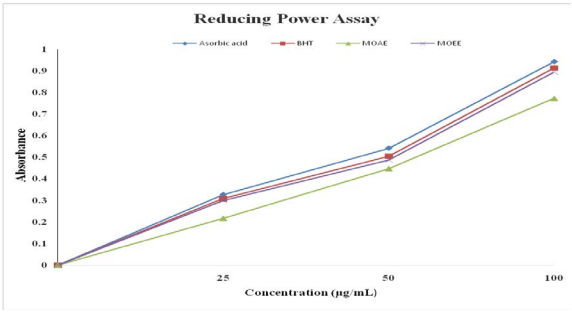

Figure 10: Comparative reducing power capacity of M. oliefera leaf exhact, ascorbic acid and butylated hydroxy toluene (BHT)

a useful indicator of antioxidant activity.[27-19] Compounds having reducing power are electron donors that can reduce oxidized intermediates in lipid peroxidation processes, allowing them to function as primary and secondary antioxidants.[28-33]

CONCLUSION

All the conducted experiments in the present research based on crude extract and are considered to be preliminary; further research is required to establish a good conclusion on the study's findings. The presence of significant quantities of phenols in *S. indicum* leaves might be a crucial predictor of antioxidant activity. These findings, together with those from prior research, show that *S. indicum* is an outstanding plant option for improving community health and nutrition, as well as a viable candidate for the development of particular nutraceuticals and bioactive products.

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