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ANTIOXIDANT AND QUALITATIVE PHYTOCHEMICAL DETERMINATION OF INDIAN SPINACH (*Basella alba*)

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Abstract
Indian spinach (Basella alba) is a widely consumed leafy vegetable known for its nutritional value and traditional medicinal applications. This study investigated the phytochemical composition and antioxidant activity of the methanolic leaf extract of Basella alba. Qualitative phytochemical screening revealed the presence of flavonoids (++), phenols, alkaloids (++), glycosides (++), terpenoids (++), saponins (++), and triterpenoids (++), while carbohydrates and anthraquinones were absent. Antioxidant activity was evaluated using the DPPH radical scavenging assay, which showed percentage inhibition ranging from 42.41% to 99.12%, with a minimum standard deviation of 0.17. The total phenolic content (TPC) was highest at 50 mg/mL (85.14%) and lowest at 10 mg/mL (37.40%). The ferric reducing antioxidant power (FRAP) assay indicated relatively low reducing capacity, although activity increased with concentration. Although the antioxidant potency of the extract was lower than standard antioxidants, the presence of bioactive phytochemicals supports the ethnomedicinal use of Basella alba as a functional food. These findings provide scientific evidence for its therapeutic potential and highlight the need for further studies to isolate active compounds and optimize extraction methods.
Keywords: *Antioxidant, phytochemicals, phenolics, medicinal plants, Basella alba*

Introduction

Many foods, such as fruits, vegetables, nuts, and whole grains, contain antioxidants. These compounds are abundant in vegetables like bell peppers, cruciferous vegetables, and leafy greens, as well as in fruits such as pomegranates, berries, and citrus fruits. Antioxidants are also present in nuts and seeds, including almonds, sunflower seeds, and pumpkin seeds. Whole grains such as brown rice, quinoa, and whole-wheat bread, as well as herbs and spices like cinnamon, ginger, and turmeric, are important sources of antioxidants.

Indian spinach makes a valuable addition to a healthy diet due to its high antioxidant content (Lolo et al., 2010; He and Pham-Huy, 2008). Certain antioxidants, such as vitamin C and beta-carotene, have the potential to reduce the risk of certain cancers.Vitamin E and other antioxidants may also help prevent heart disease by reducing inflammation and improving cholesterol levels in the body (Chandra, Pati, Phatak, and Lolo, 2010).

The phytochemical composition and antioxidant activity of Indian spinach extracts have been investigated using various methods, including spectrophotometry and high-performance liquid chromatography (HPLC) (Patel et al., 2020). These studies indicate that Indian spinach contains a wide range of phytochemicals with significant antioxidant effects. Flavonoids present in Indian spinach have been shown to possess strong anti-inflammatory and antioxidant properties, which help protect the body against oxidative stress and inflammation-related diseases. The plant is also rich in essential nutrients such as vitamins A and C, calcium, iron, and other important minerals (Kumar et al., 2019).

In traditional Ayurvedic medicine, Indian spinach leaves have been widely used for the treatment of ailments such as rheumatism, fever, and digestive disorders (Warrier et al., 2018). This study aims to address existing gaps by quantifying the antioxidant capacity of Indian spinach and identifying its phytochemical constituents. As reported by Saxena et al. (2017), the high flavonoid content in Indian spinach, particularly compounds such as quercetin and kaempferol, contributes significantly to its antioxidant properties.

Materials and methods

Sample collection

The plant material used in this study was Indian spinach (*Basella alba*). Fresh Indian spinach leaves were purchased from a local market in Garriki, Enugu, Nigeria. The leaves were cleaned with distilled water and allowed to air-dry on a clean table before being stored at 4°C in an airtight container until further analysis. Freshness, color, and texture were considered when selecting the leaves. Ten kilograms of Indian spinach leaves were collected randomly from different vendors.

Chemicals and Reagents

Solvents: Methanol (graded by HPLC) HPLC-grade acetonitrile Water (graded by HPLC) -*Criteria:* Gallic acid (Sigma-Aldrich) Ascorbic acid -*Agents of staining*: Sigma-Aldrich's 2,2-Diphenyl-1-picrylhydrazyl (DPPH) The reagent Folin-Ciocalteu (Sigma-Aldrich) -*Additional reagents*: Sigma-Aldrich's sodium carbonate and aluminum chloride Sigma-Aldrich sodium nitrite

Extraction Methods

The dried leaves were grounded and extracted using cold maceration (Harborne, 1998). The extract was filtered and used for subsequent analyses.

Determination of Total Phenolic Content

The total phenolic content (TPC) was determined using the colorimetric method based on phosphomolybdic–phosphotungstic acid reagents (Singleton and Rossi, 1999) and a standard calibration curve. A series of gallic acid standard solutions (0, 20, 40, 60, 80, and 100 µg/mL) were prepared using distilled water.

For sample preparation, an aliquot (0.5 mL) of the plant extract was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent. After 5 minutes, 2 mL of 7.5% sodium carbonate solution was added. The reaction mixture was incubated at room temperature in the dark for 30 minutes, and absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Singleton et al., 1999).

A calibration curve was constructed using the absorbance values of the gallic acid standards, and the total phenolic content was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g) (Lee et al., 2020).

Determination of DPPH Assay

The antioxidant activity of the plant extracts was evaluated using a reported method (Ronald, Prior, Xiang, and Karen, 2005). The Indian spinach extract was prepared at a concentration of 1 mg/mL in methanol. Equal volumes (100 µL each) of DPPH solution (0.1 mM in methanol) and the sample solution were mixed under identical conditions.

The mixture was incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance of the reaction mixture was measured at 517 nm using a UV-Vis spectrophotometer. The percentage inhibition of DPPH radicals was calculated using the appropriate equation.

$$\% \text{ inhibition} = (A_{control} - A_{sample}) / A_{control}) \times 100 \quad (1)$$

Determination of Ferric Reducing Power Capacity (FRAP) Assay

The Indian spinach extract solutions were prepared by dissolving the extract in distilled water to obtain a concentration of 1 mg/mL. A volume of 100 µL of FRAP reagent was added to 100 µL of the sample solution. The FRAP reagent consisted of 10 mM of 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), 40 mM HCl, and 20 mM $FeCl_3$. The reaction mixture was incubated at 37°C for 10 minutes (Benzi and Strain, 1996). Thereafter, the absorbance of the mixture was measured at 593 nm using a spectrophotometer. The FRAP value was calculated using the appropriate equation.

$$(A_{sample} - A_{blank}) / (A_{standard} - A_{blank}) \quad (2)$$

Qualitative Phytochemical Analysis

Qualitative Phytochemical analysis was carried out using a reported method (Shaikh and Patil, 2020)

Results and Discussion

The total phenolic content (TPC) of Indian spinach extracts plays a crucial role in protecting the body against oxidative damage. Phenolic compounds, which are an important class of phytochemicals, possess strong antioxidant properties and have been shown to contribute significantly to the prevention of chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders (Johnson et al., 2020).

The results obtained (Table 1, Figure 1) showed that the total phenolic content of the plant extract was highest at 50 mg/mL (85.14%), which was significantly ($p < 0.05$) higher than the values recorded at 100 mg/mL (68.84%) and 10 mg/mL (37.40%). Previous studies have indicated that the TPC of Indian spinach extracts may vary due to factors such as storage conditions, cooking methods, and environmental growth conditions (Borges et al., 2018).

For instance, a study on *Phytolacca fraternus*, a species related to Indian spinach, reported TPC values ranging from 26.69 to 61.48 mg GAE/g fresh weight. A positive correlation between antioxidant activity and TPC has also been established. Further research indicates that Indian spinach extracts exhibit antioxidant activities comparable to those of other leafy vegetables. In particular, studies have shown that the antioxidant capacity of Indian spinach is similar to that of vegetables such as kale and spinach.

Extraction studies on *Spinacia oleracea* (common spinach) reported TPC values ranging from 31.1% to 63.7%, along with notable antioxidant activity (Saini et al., 2020). Overall, the activity increases from 10 mg/mL to 50 mg/mL and then declines at 100 mg/mL, indicating that the extract does not show a strictly linear dose–response. Instead, the optimum activity occurs at 50 mg/mL, after which the effect slightly decreases. Additionally, the large standard deviation at 10 mg/mL suggests greater variability or less consistency in the extract's activity at lower concentrations.

Table 1: Total Phenolic Content (Statistical Analysis of TPC)

CONCENTRATION mg/ml	**EXTRACT**
100	**ab** **68.84±8.82**
50	b 85.14±12.95
10	a 37.40±31.90

The values are expressed as (mean±SD), n=3

Mean values with different letters of the alphabet down the column are significantly different ($p<0.05$) while mean values with same letters of the alphabet down the column are not significantly different ($p>0.05$).

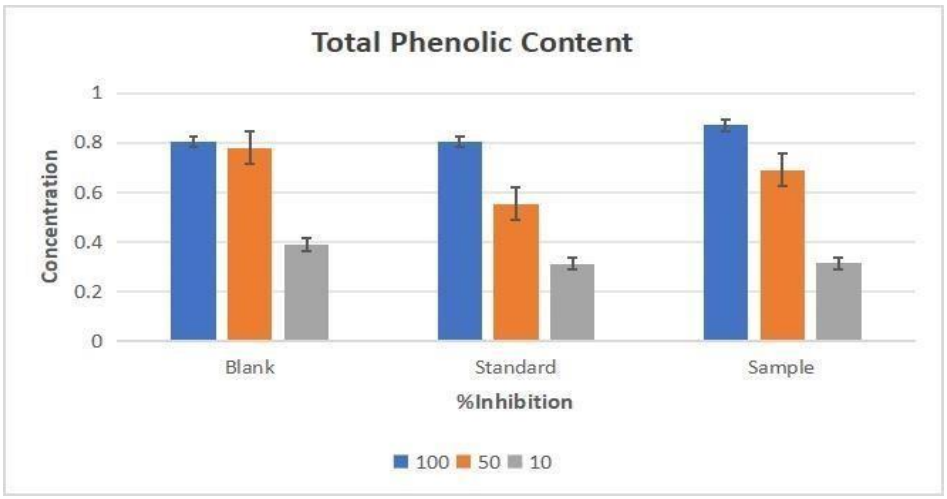


Figure 1: Total Phenolic Content

Antioxidant Assay DPPH

Table 2: Table for Antioxidant Assay

Concentration (mg/ml)	**Absorbance**	**% Inhibition**	**Standard deviation**
100	0.018	99.01%	
100	0.22	98.80	
100	0.016	99.12%	0.17
50	0.177	90.31%	
50	0.302	83.47%	
50	0.233	87.24%	3.43
10	1.052	42.41%	
10	0.743	59.34%	
10 control	1.827	0.00%	11.94

Table 2 represents the results of the antioxidant scavenging activity of the extract. Results indicate a percentage inhibition ranging from 42.41% to 99.01% across concentrations of 10–100 mg/mL, respectively. The standard deviation was less than 1 at 100 mg/mL and less than 4 at 50 mg/mL, indicating good reproducibility at higher concentrations. According to USDA (2020), one serving of cooked Indian spinach provides approximately 10% of the daily value of vitamin A, 50% of vitamin C, and 20% of folate. Additionally, it supplies about 5% calcium, 15% iron, and 10% potassium based on recommended daily values (USDA, 2020).

Patel et al. (2018) reported that Indian spinach possesses strong antioxidant activity, with an IC_{50} value of 12.50 µg/mL. This finding suggests that Indian spinach extracts can serve as effective antioxidants capable of reducing free radicals and minimizing oxidative damage. The presence of phytochemicals such as flavonoids, phenolic acids, and carotenoids contributes

significantly to the plant's anticancer and anti-inflammatory properties (Kumar et al., 2019). Indian spinach is widely consumed in traditional diets, where it is used in soups, salads, and beverages, providing essential vitamins and minerals.

The high antioxidant activity of Indian spinach extracts has been linked to their ability to prevent oxidative stress and related diseases (Zhang et al., 2020). This activity is largely attributed to the abundance of phytochemicals, including phenolic acids, flavonoids, and carotenoids. The DPPH-based IC_{50} assay remains one of the most commonly used methods for evaluating the antioxidant capacity of plant extracts. Patel et al. (2018) demonstrated the effectiveness of this method in assessing the antioxidant potential of Indian spinach, reporting an IC_{50} value of 12.50 µg/mL. These findings further support the potential of Indian spinach extracts as natural antioxidants capable of neutralizing free radicals and preventing oxidative deterioration.

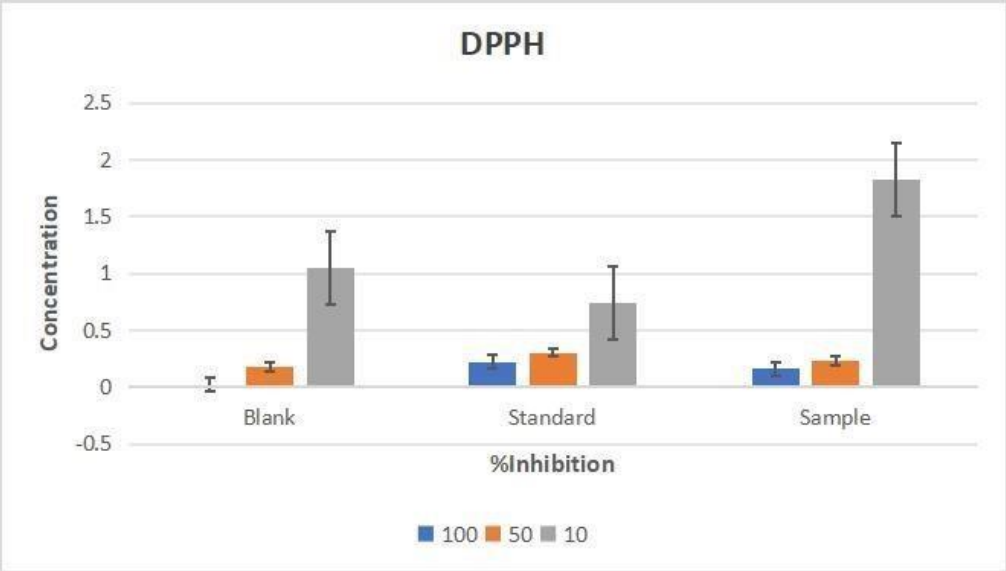


Figure 2: DPPH Assay

Statistical Analysis of the DPPH Radical Scavenging Activity

In this assay, the ability of the investigated extract to act as donors of hydrogen atoms or electrons in transformation of DPPH radical into its reduced form was investigated. The results indicated that the extract had a significant ($p < 0.05$) increase of scavenging activity in a concentration dependent manner which is comparable with the standard ascorbic acid (Table 3). There is no significant ($p > 0.05$) difference observed at concentration 100mg/ml and 50mg/ml of the extract.

Table 3: DPPH Radical Scavenging Activity (Statistical Analysis of DPPH)

CONCENTRATION mg/ml	**EXTRACT**	**STANDARD**
100	**93.95±4.73b**	**100.00±0.00c**
50	89.13±2.85^{b}	93.37±0.96^{b}
10	57.12±7.82^{a}	75.64±1.76^{a}

The values are expressed as (mean±SD), n=3

Mean values with different letters of the alphabet down the column are significantly different ($p<0.05$) while mean values with same letters of the alphabet down the column are not significantly differen**t** ($p>0.05$).

Ferric Reducing Power Capacity (FRAP) Assay

Table 4: FRAP Assay

S/N	Concentration (mg/ml)	Sample absorbance	Control absorbance	Inhibition (%)	Standard Deviation
1.	100	0.803	0.870	7.70	
2.	100	0.805	0.870	7.47	
3.	100	0.804	0.870	7.54	0.12
4.	50	0.779	0.870	–12.25	
5.	50	0.553	0.694	19.74	
6.	50	0.600	0.689	12.42	15.98
7.	10	0.389	0.314	–23.89	
8.	10	0.313	0.314	0.32	
9.	10	0.354	0.314	–12.74	12.24

Table 4 shows that the extract exhibited relatively low potency in terms of electron and/or hydrogen donating capacity, as indicated by the FRAP assay. The extract at a concentration of 100 mg/mL demonstrated significantly higher ($p < 0.05$) FRAP activity compared to concentrations of 50 mg/mL and 10 mg/mL. However, the standard antioxidant, ascorbic acid at 100 mg/mL, showed significantly higher ($p < 0.05$) activity across all concentrations evaluated. The reducing ability of the Indian spinach extract, as determined by the FRAP method, was estimated to be 3.5 mmol/g. This suggests that the extract may be useful in the treatment and prevention of oxidative stress through its ability to reduce oxidized molecules. Indian spinach extracts are known to contain high levels of phytochemicals, which are closely associated with their antioxidant properties. Johnson et al. (2019) established a clear relationship between phytochemical content and antioxidant activity in Indian spinach extracts. This highlights the critical role of phytochemicals in enhancing the antioxidant potential of the plant. Furthermore, extracts of Indian spinach have been reported to exhibit antimicrobial, anti-inflammatory, and antioxidant properties. Lee et al. (2019) noted that these biological activities may contribute to the prevention of chronic diseases. The antibacterial, anti-inflammatory, and antioxidant properties of Indian spinach extracts are therefore linked to their potential health benefits, particularly in reducing infection, inflammation, and oxidative stress. According to Zhang et al. (2020), Indian spinach extracts may offer significant health benefits by lowering the risk of chronic diseases. Existing literature on the antioxidant properties of Indian spinach is extensive and indicates promising outcomes. The medicinal value of Indian spinach can be attributed to its role as a natural source of antioxidants, owing to its rich phytochemical composition, which enables it to combat oxidative stress and related diseases.

Table 5: Ferric Reducing Power Capacity (FRAP) Assay. (Statistical Analysis of FRAP)

CONCENTRATION mg/ml	**EXTRACT**	**STANDARD**
100	**9.81±0.03c**	**66.97±4.02c**
50	9.70±0.80[b]	57.53±1.38[b]
10	9.45±0.02[a]	26.68±3.95[a]

The values are expressed as (mean±SD), n=3

Mean values with different letters of the alphabet down the column are significantly different ($p<0.05$) while mean values with same letters of the alphabet down the column are not significantly different ($p>0.05$).

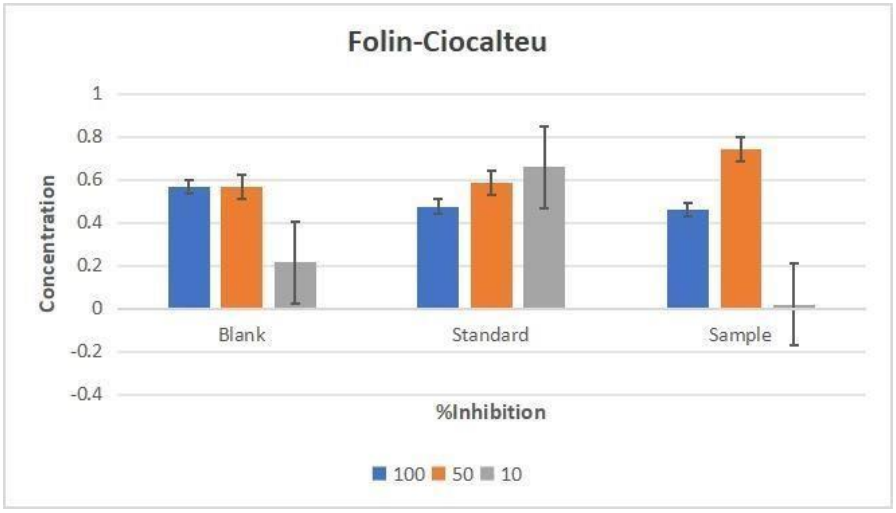


Figure 3: FRAP: Qualitative Phytochemical Analysis

Table 6**:** Result of Qualitative Phytochemical Analysis

S/N	**Parameters**	**Indian Spinach Methanol Extract**
1	Alkaloid	++
2	Carbohydrate	--
3	Protein	++
4	Flavonoid	++
5	Glycoside	++
6	Phenol	++
7	Saponin	++
8	Triterpinoid	++
9	Anthraquinone	--
10	Quinone	++
11	Cholesterol	++
12	Terpenoid	++
13	Diterpin	++

++ represents strong presence, + represents moderate presence, and - represents phytochemical's absence.

The result of the relative abundance or presence of phytochemicals in indian spinach are indicated in table 6. The Strong presence of phytochemicals (alkaloids, flavonoids, Glyhcosides, saponins, quinones, terpenoids, diterpins), Cholesterol, protein were observed. However, there were absence of carbohydrates and anthraquinones. This is similar to a reported work (Kumar *et al*., 2019).

Conclusion

This study demonstrated that the methanolic leaf extract of *Basella alba* possesses appreciable antioxidant activity and a rich phytochemical profile. Qualitative phytochemical screening confirmed the presence of key bioactive compounds, including flavonoids, phenols, alkaloids, glycosides, saponins, terpenoids, and triterpenoids, which are known contributors to antioxidant activity.

The total phenolic content (TPC) showed a concentration-dependent trend, with the highest value observed at 50 mg/mL (85.14%), followed by 100 mg/mL (68.84%) and 10 mg/mL (37.40%). This indicates that phenolic compounds are optimally expressed at moderate concentrations, with a decline at higher concentrations possibly due to aggregation or assay interference.

The DPPH radical scavenging assay revealed strong antioxidant activity, with percentage inhibition ranging from 42.41% at 10 mg/mL to over 99% at 100 mg/mL, indicating a clear concentration- dependent increase in free radical scavenging ability. However, no significant difference was observed between 50 mg/mL and 100 mg/mL, suggesting a possible saturation effect at higher concentrations.

In contrast, the FRAP assay indicated relatively low reducing power of the extract compared to the standard antioxidant, although a slight increase in activity was observed with increasing concentration. This suggests that while the extract is effective in radical scavenging, its electron-donating capacity is comparatively limited.

Overall, the antioxidant activity observed across the assays correlates with the presence of phenolic compounds and other phytochemicals in the extract. These findings support the traditional use of *Basella alba* as a medicinal plant and highlight its potential as a natural source of antioxidants for nutraceutical and pharmaceutical applications. Further studies are recommended to isolate and characterize the specific bioactive compounds responsible for these activities and to optimizeextraction conditions for enhanced efficacy.

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