

## Phytochemical screening and antioxidant activity of *Ipomoea hederifolia* stems: A potential medicinal plant

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**Abstract.** Hossain MM, Uddin MS, Baral PK, Ferdus M, Bhowmik S. 2022. Phytochemical screening and antioxidant activity of *Ipomoea hederifolia* stems: A potential medicinal plant. *Asian J Nat Prod Biochem* 20: 41-47. *Ipomoea hederifolia* L., a plant of the Convolvulaceae family, popularly known as morning glory, possesses numerous medicinal values. The present study aimed to explore the antioxidant activity and bioactive compounds of *I. hederifolia* stems (IHS). The IHS was soaked in methanol for 21 days. The filtrate was concentrated using a rotary evaporator after filtration to obtain IHS extract, which is subjected to phytochemical screening using various tests. Antioxidant activity was determined using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and reducing power activity (RPA) methods. The phytochemical screenings revealed that IHS extract possesses carbohydrates, tannins, flavonoids, phenols, saponins, alkaloids, steroids, anthraquinones, and cardiac glycosides. The antioxidant activity of IHS extract in DPPH model was moderate (IC<sub>50</sub>: 174.08 µg/ml) in comparison with standards (IC<sub>50</sub>: 102.28 µg/mL AA; IC<sub>50</sub>: 88.52 µg/mL for BHT). It indicates that IHS exerts free radical scavenging power in a dose-dependent fashion. The RPA model also produced moderate antioxidant potential (EC<sub>50</sub>: 279.58 µg/mL for IHS; EC<sub>50</sub>: 23.121 µg/mL for AA; EC<sub>50</sub>: 50.84 µg/mL for BHT) depending on increasing order of dose. Based on the findings of this investigation, we can conclude that IHS extract possesses various bioactive compounds and moderate antioxidant potentials, which may be a path to the discovery of traditional medicines and remedies for many critical diseases.

**Keywords:** Antioxidant activity, DPPH, *Ipomoea hederifolia*, phytochemical screening, RPA

**Abbreviations:** AA: Ascorbic acid; BHT: butylated hydroxyl toluene; DPPH: 1,1-Diphenyl-2-picrylhydrazyl; EC<sub>50</sub>: Half maximal effective concentration; IC<sub>50</sub>: Half maximal inhibitory concentration; IHS: *Ipomoea hederifolia* stems; RPA: Reducing power activity; WHO: World Health Organization

### INTRODUCTION

Nature has been regarded as the greatest source of medicinal agents for thousands of years (Uddin et al. 2019). A huge number of modern drugs have been isolated from natural sources based on their use in traditional medicine (Millat et al. 2019; Kurian et al. 2022). Many important pharmaceuticals that indigenous people currently use originated from plant sources (Balick et al. 1996). The developing countries mostly rely on traditional plants and focus on healthcare applications (Allameh et al. 2002). Traditional medicine involves using different plant extracts or bioactive constitutions (Davis et al. 1994).

Several edible plants play a vital role in different ethnic groups by providing fresh food, nutrition, and medicines. Various research has been conducted to record ethnobotanical knowledge of wild edible plant species (Mallick et al. 2020). Many plant species have demonstrated significant pharmacological activities in animal and human studies, such as antioxidant, cytotoxic, anti-inflammatory, antiproliferative, analgesic, immunomodulatory, antimicrobial, hepatoprotective, hypoglycemic, hypotensive, hypolipidemic, diuretic, etc. (Nugroho et al. 2020; Putra et al. 2020; Ray and Saini

2021). In addition, these plants have abundant flavonoids, alkaloids, phenols, saponins, anthraquinone, tannins, cardiac glycosides, steroids, etc. These bioactive compounds and therapeutic actions verify many folk medicinal claims on edible plant species.

About 64% of the global population remains dependent on traditional medicines and medicinal plants to provide for their healthcare needs (Cotton 1996). According to a study by WHO, practitioners are prescribing traditional medicine to about 80% of patients in India, 85% in Burma, and 90% in Bangladesh. The writings indicate that the therapeutic use of plants is as old as 4000-5000 BC, and the Chinese first used natural herbal preparation as medicines. Also, modern pharmacopeia still contains at least 25% of drugs derived from plants and many other synthetic compounds isolated from plants (Tewari 2000). Since disease, decay, and death have always co-existed with life, early man had to think about disease and its treatment. Thus, the human race started using plants to treat diseases and injuries from the early days of civilization on earth. Its long journey from ancient to modern times has successfully been an effective therapeutic tool for fitting against diseases and various health hazards (Ghani 1998).

The human body possesses both enzymatic and non-enzymatic antioxidant defenses to combat free radicals and other oxidants (Alam et al. 2013). Free radicals cause cancers, Parkinson's, cardiovascular disease, Alzheimer's, neural disorders, mild cognitive impairment, ulcerative colitis, and alcohol-induced liver disease (Alam et al. 2013). Antioxidants boost protection against free radicals. Substantial data suggest that antioxidant-rich diets may be important in disease prevention. Scientists agree that a mixture of antioxidants may be more beneficial for long-term immunity boosting. Synthetic antioxidants have been seriously explored for animal use to enhance health, performance, and product quality. Possibly these substances might cause harm; thus, their innocuousness must be questioned. Many phytochemicals have been found to benefit animals in terms of improved performance and quality (Ansari et al. 2012; Lee et al. 2013), as well as an improved endogenous antioxidant system (Aggarwall and Shishodi 2006), potentially by impacting particular molecular targets directly or indirectly through stabilized conjugates that influence metabolic pathways. Therefore, scientists are searching for new antioxidant compounds to address various diseases.

*Ipomoea hederifolia* L. (Convolvulaceae), known as morning glory, is an annual twiner with a thin and frail stem (Flora of Bangladesh 2022). It is typically found in the untamed areas of the Chattogram Hill Tracts in Bangladesh. According to India's indigenous medicine system, it belongs to anti-psychotic, antioxidant, anti-cancer, antimicrobial, oxytocic, and anti-inflammatory activities. However, no ethnopharmacological study has been conducted on its stem. Therefore, the present study aimed to explore the antioxidant activity and bioactive compounds of *I. hederifolia* stems (IHS).

## MATERIALS AND METHODS

### Plant collection

IHS was collected from the Baroiyadhala National Park in Chittagong, Bangladesh. First, the stems were handpicked from healthy plants and washed with distilled water. Then stems were subjected to sun drying for two weeks. Finally, dried stems were powdered into a fine powder and stored in an airtight container.

### Extract preparation

About 200 gm of powdered stems were soaked for 21 days in 1000 mL methanol to dissolve the bioactive compounds in a solvent. Then the soaked powder was filtrated with Whatman filter paper. Finally, the filtrate was collected and subjected to a rotary evaporator to obtain a concentrated extract (Millat et al. 2019; Naznin et al. 2019).

### Phytochemical screening

The preliminary phytochemical tests determine the presence of several chemical groups in the extract. A tiny amount of methanol extract of IHS was subjected to preliminary qualitative phytochemical evaluation using

recognized methodologies for detecting phytochemicals such as alkaloids, cardiac glycosides, anthraquinone, steroids, and saponins (Uddin et al. 2020; Talukder et al. 2022). The detection was based on visual observations of a color change or precipitate formation after adding specific reagents.

### Carbohydrate screening

**Benedict's test:** In test tubes, a few drops of Benedict's reagent were added to 1 mL of extract and heated for a few minutes in a water bath. The presence of carbohydrates in the extracts is indicated by the formation of a reddish-brown precipitate in the tube. **Molisch's Test:** 1 mL of extract was mixed with 1 mL of Molisch's reagent and a few drops of concentrated  $H_2SO_4$ . The presence of carbohydrates in the extracts is shown by the formation of a purple or red color in the tube (Sheela Rani et al. 2013).

### Tannin screening

In tubes, 1 mL of the extract was mixed with 2 mL of 5% ferric chloride. The presence of tannins in the extract is shown by the formation of greenish-black or dark blue in the tube. The extract was dissolved in 10 mL of distilled water and then filtered. About 1% aqueous solution of  $FeCl_3$  was also added. The presence of tannins in the extracts is indicated by the formation of strong green, purple, blue, or black in the tube (Ögenler et al. 2018).

### Saponins screening

Two (2) mL of distilled water were added to the 2 mL extract and thoroughly mixed for 15 minutes by shaking lengthwise in a graduated cylinder. The presence of saponins in the extracts is shown by forming a 1 cm layer of foam (Sofowora et al. 2013).

### Flavonoid screening

A total of 0.5 g of extract was thoroughly mixed with petroleum ether (lipid layer) to eliminate the fatty components. Then, the defatted residue was filtered after being dissolved in 20 mL of 80% ethanol. The filtrate was used in the following experiments:

In a test tube, 3 mL of the filtrate was combined with 4 mL of 1% aluminum chloride in methanol. The formation of yellow color indicates the presence of flavonols, flavones, and chalcones in the extracts. Dilute ammonia solution (5 mL) was added to a fraction of each plant extract's aqueous filtrate, followed by adding conc.  $H_2SO_4$ . The presence of flavonoids in the extracts is shown by the formation of yellow color (Yusuf et al. 2014).

**Shinoda's Test:** The extract was first dissolved in alcohol, and then a fraction of magnesium was combined with conc. HCl, which was applied dropwise. The presence of flavonoids in the extracts is shown by the formation of a magenta color (Hossain et al. 2013).

### Alkaloid screening

**Mayer's test:** 2 mL of the stem extract was mixed with 2 mL of concentrated hydrochloric acid (HCl). A few drops of Mayer's reagent were then added. The presence of

alkaloids in the extracts is indicated by forming a green or white precipitate (Polyum and Phinthida 2018).

**Wagner's Test:** Filtrates were subjected to Wagner's reagent treatment (Iodine in Potassium iodide). The formation of brown/reddish precipitate revealed alkaloids' presence.

**Dragendroff's Test:** Dragendroff's reagent was used to treat the filtrates (solution of Potassium Bismuth Iodide). The formation of an orange-red precipitate showed the presence of alkaloids.

### Quinine screening

An alcoholic potassium hydroxide solution was added to 1 mL of various extracts. The color change shows the presence of quinines in the extracts from red to blue. One (1) mL of conc.  $H_2SO_4$  was added to 1 mL of the various extracts. The presence of quinones in the extracts is shown by the formation of a red hue in the tube (Zohra et al. 2012).

### Tarpinoid screening

**Salkowski Test:** Solvent extract (5 mL) was combined with chloroform (2 mL) and saturated  $H_2SO_4$  (3 mL). A layer of reddish brown color appeared at the tube interface, indicating the presence of terpenoids in the extracts (Zohra et al. 2012).

### Glycoside screening

**Keller Killiani Test:** The extract was treated with a few drops of glacial acetic acid and a ferric chloride solution. The tube was well mixed by vigorous shaking, and then conc.  $H_2SO_4$  was added. In the tube, two layers can be seen: the reddish brown layer in the lower section and the acetic acid layer in the upper part. Subsequently, the presence of glycosides in the extracts shows the solution turning bluish-green (Mulla and Paramjyothi 2010).

**Bromine water test:** A few drops of bromine water were added to the extract. The presence of glycosides in the extracts is shown by forming a yellow precipitate in the test tube (Mulla and Paramjyothi 2010).

### Anthraquinone glycosides

**Borntrager's test:** Plant extract of 100 mg was mixed in 5 mL of chloroform and filtered. The filtrate was then thoroughly agitated with an equal amount of ammonia solution (10%  $NH_4OH$ ). The presence of anthraquinones is indicated by the emergence of a pink-violet or red color in the ammoniacal layer (Zohra et al. 2012).

### Triterpenoid screening

**Liebermann Burchard test:** A few drops of acetic anhydride were added to the extract, and the mixture was heated and cooled. Along the walls of the test tube, conc.  $H_2SO_4$  was added. The presence of triterpenoids in the extracts is indicated by the formation of a brown ring at the intersection of two layers, deep red color in the bottom half and green color in the upper part (Francis and Suseem 2016).

### Phenol screening

**Ferric chloride test:** 50 mg extract was thoroughly mixed with distilled water before adding a few drops of 5%  $FeCl_3$  solution. The presence of phenols is indicated by the formation of blue, green, and violet colors.

**Gelatin test:** A little amount of extract was thoroughly mixed with distilled water, and 2 mL of a 1% gelatin solution containing 10% NaCl was added and mixed thoroughly. The presence of phenols in the extracts is shown by forming a white precipitate (Sheela Rani et al. 2013).

### Cardiac glycoside screening

In a test tube, 2 mL of the mother solution of extracts was mixed with a few drops of weak hydrochloric acid, 2 mL of Sodium Nitroprusside in pyridine, and 2 mL of Sodium Hydroxide solution. The presence of cardiac glycosides in the extracts is indicated by the formation of a pink to blood-red color (Zohra et al. 2012).

### Steroid screening

**Salkowski's test:** 1 mL of each extract was mixed with 1mL chloroform and a few drops of concentrated  $H_2SO_4$ . The presence of steroids in the extracts is shown by forming a brown ring (Oyinlade 2014).

**Liebermann Burchard's test:** Chloroform extracts were treated with acidic anhydride, heated, cooled, and then treated with strong sulfuric acid. The appearance of a transitory greenish tint indicates the presence of steroids (Raaman 2006).

### Antioxidant assay

#### DPPH method

The antioxidant activity of the methanol extract of IHS was determined using the scavenging activity of the stable 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical, as described by Khanapur et al. (2014) with minor alterations. At first, a fresh 0.2 mM DPPH solution in methanol was prepared. 2 mL of each extract, Ascorbic acid, and Butylated hydroxytoluene solution were pipetted into separate test tubes, and 3 mL of 0.2 mM DPPH solution was added to each sample to initiate the reaction. All of the samples were mixed and kept in a dark place for 30 minutes. After 30 minutes of incubation, the absorbance was measured using a UV-VIS Spectrophotometer at 517 nm. Methanol was utilized as a control, while a 0.2 mM DPPH solution was used as a blank. All extracts and standards were analyzed in triplicate. The antioxidant activity of each extract was measured by estimating the percentage of antioxidant activity using the following formula based on the reduction of DPPH absorbance.

$$\text{Percentage (\%)} \text{ of free radical DPPH scavenging} = \frac{(A_0 - A_1)}{A_0} \times 100.$$

Where;  $A_0$  is the absorbance of the control solution, which contains all reagents except plant extracts, and  $A_1$  is the absorbance of the DPPH solution, which contains plant extracts. Finally, the concentration of sample necessary to scavenge 50% of the DPPH free radical ( $IC_{50}$ ) was

computed using the plot of the percentage of inhibition versus extract concentration. For this experiment, ascorbic acid and BHT were utilized as standards.

#### Reducing power activity (RPA)

The reducing power of the plant extracts, ascorbic acid, and BHT was determined using the method reported by Kubola and Siriamornpun (2008) with minor modifications. Ascorbic acid and BHT were employed as standards in this study. A variety of ascorbic acid, BHT, and IHS solutions was created. An aliquot of the varying concentrations of the standard and sample solution reagents was added, followed by 2.5 mL of sodium phosphate buffer and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated in a water bath at 50°C for 20 minutes before being cooled and terminated (reaction) with 2.5 mL of 10% (w/v) trichloroacetic acid. After centrifuging the mixture at 3000 rpm (930 x g) for 10 minutes, 2.5 mL of the supernatant was combined with 2.5 mL of distilled water and 0.5 mL of freshly made 0.1% (w/v) anhydrous ferric chloride (FeCl<sub>3</sub>) solution. A UV-1601 Shimadzu UV-Vis spectrophotometer was used to measure absorbance at 700 nm. A linear connection was created after graphing the absorbance against the concentration, which was utilized as a standard curve for determining the RPA of the test samples. The results were presented as effective concentration (EC<sub>50</sub>) values, and the RPA was calculated using a standard curve.

#### Statistical analysis

In this investigation, the findings were summarized using the mean value and the standard deviation of at least three independent samples. Statistical analyses were conducted utilizing IBM's Statistical Package for the Social Sciences (SPSS, Version 24).

## RESULTS AND DISCUSSION

The phytochemical screening of IHS showed the presence of alkaloids, cardiac glycosides, flavonoids, steroids, saponin, diterpenes, tannins, carbohydrates, and phenols, as exhibited in Table 1. IHS extract was subjected to assessment for antioxidant activity based on these results. The study showed that the plant could be used for various purposes.

The free radical scavenging and antioxidant activity of medicinal plants are linked with the remedy of several diseases (Prathapan et al. 2011). The DPPH test demonstrates a substance's antioxidant activity by causing a color change when placed in an environment containing a free radical scavenger (Naznin et al. 2019). The reaction that causes the color to change from purple to yellow indicates the process by which the DPPH radical acts as the oxidizing radical that the antioxidant must reduce. The presence of an odd electron in DPPH is responsible for the color transformation and absorbance that can be seen at 517 nm (Naznin et al. 2019). The antioxidant activity of

IHS was shown by its methanolic extract with DPPH, which reduced DPPH-H. The degree of discoloration that occurred was used to measure the antioxidant activity. The extracts showed a proclivity to eliminate DPPH free radicals in this study, with an IC<sub>50</sub> value higher than the reference standards AA and BHT (Table 2). The IC<sub>50</sub> values of AA, BHT, and IHS were 102.28, 88.52, and 174.08 µg/mL, respectively. The maximum % of inhibition for AA, BHT and IHS were 91.77%, 88.30 % and 88.82% at 500 µg/mL where the minimum % of inhibition were 19.28%, 18.12% and 6.43% at 0.977 µg/mL, respectively. That indicates that the DPPH radical activity of methanol IHS extracts increased dose-dependent (Figure 1). These results collectively reported that IHS possesses a strong scavenging activity compared to standards.

Another important method for the evaluation of antioxidant activity is the RPA method. A compound's reducing capability may indicate its antioxidant potential (Meir et al. 1995). The reduction of Fe<sub>3</sub><sup>+</sup> is widely employed as an indication of electron-donating activity, where the solution's yellow color changes to blue and green depending on a compound's ability to reduce free radicals. Phenols convert ferric–ferricyanide to prussian blue ferrocyanide. In this study, the maximum absorbance for BHT, AA, and IHS were 2.523, 4.12, and 0.775 at 500 µg/mL, whereas the minimum absorbances were 0.192, 0.183, and 0.175 at 1.953 µg/mL respectively (Table 3). Again, the EC<sub>50</sub> value for BHT, AA, IHS were 50.84, 23.12, and 279.58 µg/mL respectively. That indicates that IHS possesses moderate antioxidant capacity as compared to standards.

**Table 1.** Bioactive compounds analysis of methanol extract of IHS

| Constituents       | Tests                               | Methanol extract of IHS |
|--------------------|-------------------------------------|-------------------------|
| Alkaloids          | Mayer's reagent                     | +                       |
|                    | Wagner's reagent                    | +                       |
|                    | Dragendoff's reagent                | +                       |
| Cardiac glycosides | Keller-Killiani Test                | +                       |
| Steroids           | Salkowski's Test                    | +                       |
|                    | Lieberman-Burchard test             | +                       |
| Saponins           | Frothing test                       | +                       |
| Anthraquinones     | Borntrager's test                   | -                       |
| Carbohydrates      | Benedict's test                     | +                       |
|                    | Molisch's Test                      | +                       |
| Tannin             |                                     | +                       |
| Flavonoid          | Elimination of the fatty components | +                       |
|                    | Shinoda's Test                      | +                       |
|                    | Salkowski Test                      | -                       |
| Tarpinoid          | Lieberman-Burchard test             | -                       |
| Phenols            | Ferric chloride test                | +                       |
|                    | Gelatin test                        | +                       |

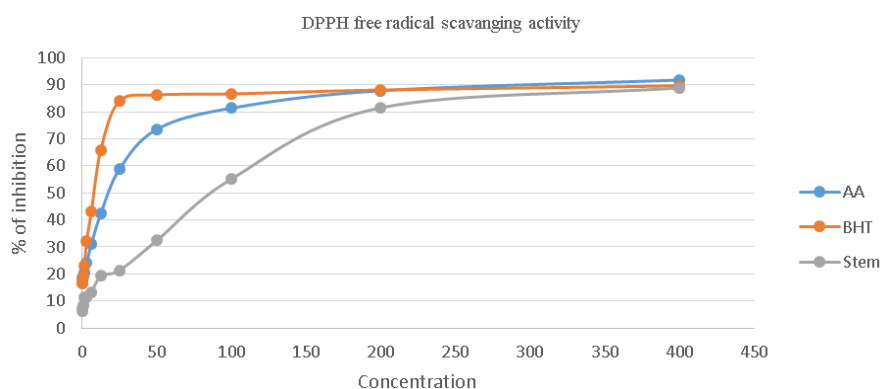
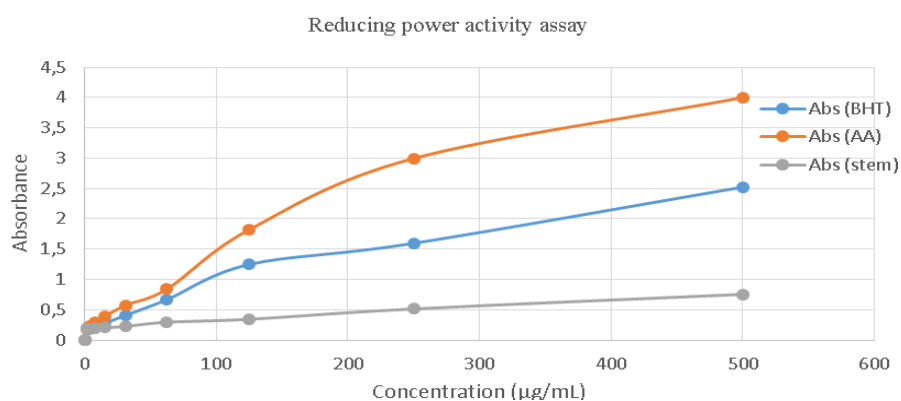
Note: (+) Indicates the presence and (-) indicates the absence of a chemical compound in the sample

**Table 2.** DPPH free radical scavenging (SCV) activity of methanol extract of the IHS, ascorbic acid (AA), and butylated hydroxyl toluene (BHT)

| Conc.<br>( $\mu\text{g/mL}$ ) | Avg. absorbance   |                   |                   | % of inhibition |       |       | IC <sub>50</sub> ( $\mu\text{g/mL}$ ) |       |        |
|-------------------------------|-------------------|-------------------|-------------------|-----------------|-------|-------|---------------------------------------|-------|--------|
|                               | AA                | BHT               | IHS               | AA              | BHT   | IHS   | AA                                    | BHT   | IHS    |
| 0                             | 0.778 $\pm$ 0.002 | 0.778 $\pm$ 0.005 | 0.778 $\pm$ 0.016 | 0.0             | 0.0   | 0.0   | 102.28                                | 88.52 | 174.08 |
| 0.97656                       | 0.628 $\pm$ 0.042 | 0.637 $\pm$ 0.015 | 0.728 $\pm$ 0.005 | 19.28           | 18.12 | 6.43  |                                       |       |        |
| 1.9531                        | 0.619 $\pm$ 0.088 | 0.603 $\pm$ 0.002 | 0.712 $\pm$ 0.002 | 20.44           | 22.49 | 8.48  |                                       |       |        |
| 3.90625                       | 0.589 $\pm$ 0.032 | 0.561 $\pm$ 0.003 | 0.689 $\pm$ 0.012 | 24.29           | 27.89 | 11.44 |                                       |       |        |
| 7.8125                        | 0.536 $\pm$ 0.025 | 0.509 $\pm$ 0.008 | 0.675 $\pm$ 0.007 | 31.11           | 34.56 | 13.24 |                                       |       |        |
| 15.625                        | 0.449 $\pm$ 0.032 | 0.418 $\pm$ 0.014 | 0.628 $\pm$ 0.004 | 42.29           | 46.27 | 19.28 |                                       |       |        |
| 31.25                         | 0.321 $\pm$ 0.015 | 0.266 $\pm$ 0.018 | 0.562 $\pm$ 0.016 | 58.74           | 65.81 | 27.76 |                                       |       |        |
| 62.5                          | 0.206 $\pm$ 0.077 | 0.159 $\pm$ 0.003 | 0.387 $\pm$ 0.008 | 73.52           | 79.56 | 50.26 |                                       |       |        |
| 125                           | 0.145 $\pm$ 0.006 | 0.125 $\pm$ 0.012 | 0.193 $\pm$ 0.014 | 81.36           | 83.93 | 75.19 |                                       |       |        |
| 250                           | 0.095 $\pm$ 0.003 | 0.104 $\pm$ 0.025 | 0.135 $\pm$ 0.024 | 87.78           | 86.63 | 82.65 |                                       |       |        |
| 500                           | 0.064 $\pm$ 0.012 | 0.091 $\pm$ 0.032 | 0.087 $\pm$ 0.005 | 91.77           | 88.30 | 88.82 |                                       |       |        |

**Table 3.** Reducing power activity of IHS, AA, and BHT

| Concentration<br>( $\mu\text{g/mL}$ ) | Avg. absorbance   |                   |                   | EC <sub>50</sub> ( $\mu\text{g/mL}$ ) |        |        |
|---------------------------------------|-------------------|-------------------|-------------------|---------------------------------------|--------|--------|
|                                       | BHT               | AA                | IHS               | BHT                                   | AA     | IHS    |
| 1.953125                              | 0.192 $\pm$ 0.004 | 0.183 $\pm$ 0.024 | 0.175 $\pm$ 0.001 | 50.84                                 | 23.121 | 279.58 |
| 3.90625                               | 0.204 $\pm$ 0.026 | 0.222 $\pm$ 0.002 | 0.185 $\pm$ 0.022 |                                       |        |        |
| 7.8125                                | 0.258 $\pm$ 0.034 | 0.299 $\pm$ 0.008 | 0.192 $\pm$ 0.014 |                                       |        |        |
| 15.625                                | 0.286 $\pm$ 0.008 | 0.393 $\pm$ 0.012 | 0.205 $\pm$ 0.001 |                                       |        |        |
| 31.25                                 | 0.41 $\pm$ 0.043  | 0.576 $\pm$ 0.004 | 0.226 $\pm$ 0.005 |                                       |        |        |
| 62.5                                  | 0.668 $\pm$ 0.052 | 0.84 $\pm$ 0.001  | 0.295 $\pm$ 0.024 |                                       |        |        |
| 125                                   | 1.247 $\pm$ 0.038 | 1.82 $\pm$ 0.043  | 0.344 $\pm$ 0.012 |                                       |        |        |
| 250                                   | 1.597 $\pm$ 0.004 | 2.996 $\pm$ 0.028 | 0.514 $\pm$ 0.008 |                                       |        |        |
| 500                                   | 2.523 $\pm$ 0.006 | 4.12 $\pm$ 0.001  | 0.755 $\pm$ 0.004 |                                       |        |        |

**Figure 1.** DPPH free radical scavenging activity assay of methanol extract of IHS in comparison with ascorbic acid and butylated hydroxyl toluene**Figure 2.** Reducing power activity assay of methanol extract of IHS in comparison with AA and BHT as standards

Since the dawn of civilization, people have relied on plants as a source of medicine to treat a wide variety of illnesses. Currently, the study of phytopharmacology has opened up a new field for discovering plant-derived pharmaceuticals that are helpful for the treatment of certain disorders and attract the attention of those working in the field of herbal medicine. Around thirty percent of medications are believed to be generated from plant compounds (Uddin et al. 2020). Our study has identified the presence of bioactive compounds such as alkaloids, cardiac glycosides, flavonoids, steroids, saponins, tannins, and phenols in the *I. hederifolia* stem. These bioactive compounds are responsible for curing tons of diseases. Several studies revealed that phenols and flavonoids are phytochemicals that exert strong antioxidant activity (Shi et al. 2003; Brenes et al. 2008; Lee et al. 2017). Our preliminary phytochemical screening showed the presence of phenols and flavonoids in the *I. hederifolia* stem. Therefore, we can claim that the *I. hederifolia* stem belongs to good antioxidant activity. We hope researchers will isolate new pharmacologically active compounds based on our current investigation.

In conclusion, the findings of this research demonstrate that IHS contains alkaloids, cardiac glycosides, flavonoids, steroids, saponin, diterpenes, tannins, carbohydrates, and phenols, which are powerful phytochemicals. Moreover, the current investigation also found that IHS had modest antioxidant activity compared to a reference standard. Therefore, a high concentration of phenolic compounds, as well as other phytochemicals, might be linked with the plant's antioxidant effects. So, extracting the plant's bioactive components might be a viable alternative to synthetic antioxidants.

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