

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 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 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, saponin, alkaloids, steroids, anthraquinones, and cardiac glycosides. The antioxidant activity of IHS extract in DPPH model was moderate (IC₅₀: 174.08 µg/ml) in comparison with standards (IC₅₀: 102.28 µg/mL AA; IC₅₀: 88.52 µg/mL for BHT). It indicates that IHS exerts free radical scavenging power in dose dependent fashion. The RPA model also produced moderate antioxidant potential (EC₅₀: 279.58 µg/mL for IHS; EC₅₀: 23.121 µg/mL for AA; EC₅₀: 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 path to 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; EC₅₀: Half maximal effective concentration; IC₅₀: Half maximal inhibitory concentration; IHS: *Ipomoea hederifolia* stems; RPA: Reducing power activity; WHO: World Health Organization

INTRODUCTION

Nature is regarded as the greatest source of medicinal agents for thousands 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 which are currently being used by indigenous people, originated from plant source (Balick et al. 1996). The developing countries are mostly relying on traditional plants and focusing towards healthcare applications (Allameh et al. 2002). The traditional medicine involves the use of different plant extracts or the 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 researches have 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, anti-proliferative, analgesic, immunomodulatory, antimicrobial, hepatoprotective, hypoglycemic, hypotensive, hypolipidemic, diuretic, etc

(Nugroho et al 2020; Putra et al. 2020; Ray and Saini 2021). Flavonoids, alkaloids, phenols, saponins, anthraquinone, tannins, cardiac glycosides, steroids, etc. are abundant in these plants. These bioactive compounds and therapeutic actions verify many folk medicinal claims on edible plant species.

About 64% of the total global population remains dependent on traditional medicines and medicinal plants for provision of their health-care needs (Cotton 1996). According to a study of WHO, the practitioners are prescribing traditional medicine about 80% of patients in India, 85% in Burma and 90% in Bangladesh. The writings indicate that therapeutic use of plants is as old as 4000-5000 B.C. and Chinese used first the natural herbal preparation as medicines. Also, modern pharmacopoeia still contain at least 25% drugs derived from plants and many others which are synthetic compounds that were isolated from plants (Tewari 2000). Since disease, decay and death have always co-existed with life, the early man had to think about disease and its treatment. Thus the human race started using plants as a means of treatment of diseases and injuries from the early days of civilization on earth and its long journey from ancient time to modern time

has successfully as effective therapeutic tools 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 disease, cardiovascular disease, Alzheimer's disease, neural disorders, mild cognitive impairment, ulcerative colitis and alcohol-induced liver disease (Alam et al. 2013). Antioxidants boost protection against free radicals. Substantial data suggests 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 use in animals to enhance health, performance, and product quality. It is possible that 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), popularly 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. It belongs to anti-psychotic, anti-oxidant, anti-cancer, anti-microbial, oxytocic and anti-inflammatory activities according to indigenous medicine system in India. However, no ethno-pharmacological 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 were collected from the Baroiyadhala National Park in Chittagong, Bangladesh. The stems were handpicked from a healthy plants and washed with distilled water. Then stems were subjected to sun dry for two weeks. Finally, dried stems were powdered into a fine powders and stored in an airtight container.

Extract preparation

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

Phytochemical screening

The preliminary phytochemical tests consist of determining 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 the detection of 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 following the addition of specific reagents.

Carbohydrate screening

Benedict's test: In a 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 (Sheela Rani et al. 2013).

Molish's Test: 1 mL of extract was mixed with 1 mL of Molish's reagent and a few drops of concentrated H₂SO₄. The presence of carbohydrates in the extracts is shown by the formation of purple or red color in the tube (Sheela Rani et al. 2013).

Tannin screening

In a 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 FeCl₃ 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 was added to the 2 mL of extract and thoroughly mixed for 15 minutes by shaking lengthwise in a graduated cylinder. The presence of saponins in the extracts is shown by the formation of a 1 cm layer of foam (Sofowora et al. 2013).

Flavonoid screening

To eliminate the fatty components, the 0.5 g of extract was thoroughly mixed with petroleum ether (lipid layer). 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 presence of flavonols, flavones, and chalcones in the extracts is indicated by the formation of yellow color.

Dilute ammonia solution (5 mL) was added to a fraction of each plant extract's aqueous filtrate, followed by the addition of conc. H₂SO₄. 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 magenta color (Hossain et al. 2013).

Alkaloid screening

Mayer's test: 2 mL of the stems 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 the formation of green or white precipitate (Polyium and Phinthida 2018).

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

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

Quinine screening

An alcoholic potassium hydroxide solution was added to 1 mL of various extracts. The presence of quinines in the extracts is shown by the color change from red to blue.

One (1) mL of conc. H₂SO₄ 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 H₂SO₄ (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: Treatment of the extract was done with a few drops of glacial acetic acid and a solution of ferric chloride. The tube was well mixed by vigorous shaking, and then conc. H₂SO₄ 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 is shown by 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 the formation of yellow precipitate in the test tube (Mulla and Paramjyothi 2010).

Anthraquinone glycosides

Bortrager's test: Plant extract of 100 mg were mixed in 5 mL of chloroform and filtered. The filtrate was then thoroughly agitated with an equal amount of ammonia solution (10% NH₄OH). The presence of anthraquinones is indicated by the emergence of 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. H₂SO₄ 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% FeCl₃ 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 the formation of white precipitate (Sheela Rani et al. 2013).

Cardiac glycoside screening

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

Steroid screening

Salkowski's test: 1 mL of each extract was mixed with 1 mL of chloroform and a few drops of concentrated H₂SO₄. The presence of steroids in the extracts is shown by the formation of 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 greenish transitory tint indicates the presence of steroids (Raaman 2006).

Antioxidant assay

DPPH method

The antioxidant activity of 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 hydroxy toluene 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 together and kept in the 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 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 modification. Ascorbic acid and BHT were employed as standards in this study. A variety of ascorbic acid, BHT, and IHS solutions were created. An aliquot of the varied 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 ($FeCl_3$) 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_{50}) values, and the RPA was calculated using standard curve.

Statistical analysis

In this investigation, the findings were summarized using the mean value together with 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 which is exhibited in Table 1. Based on these results, IHS extract was subjected to assess for antioxidant activity. The study showed that the plant can be used for various purposes.

The free radical scavenging and antioxidant activity of medicinal plants are linked with the remedy of a number of diseases (Prathapan et al. 2011). The DPPH test demonstrates a substance's antioxidant activity by causing a change in color 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 is an indicator of the process by which the DPPH radical acts as the oxidizing radical that must be reduced by the antioxidant. The presence of an odd electron in DPPH is

responsible for the transformation in color, as well as the 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 resulted in the reduction of DPPH-H. The degree of discoloration that occurred was used as a measurement of the antioxidant activity. In this study, the extracts shown a proclivity to eliminate DPPH free radicals, with an IC_{50} value higher than that of the reference standards AA and BHT (Table 2). The IC_{50} values of AA, BHT and IHS were 102.28, 88.52 and 174.08 $\mu\text{g/mL}$ respectively. The maximum % of inhibition for AA, BHT and IHS were 91.77%, 88.30 % and 88.82% at 500 $\mu\text{g/mL}$ where the minimum % of inhibition were 19.28%, 18.12% and 6.43% at 0.977 $\mu\text{g/mL}$, respectively. This indicate that the DPPH radical activity of methanol IHS extracts increased at dose dependent fashion (Figure 1). Collectively, these results reported that IHS possesses a strong scavenging activity as compared to standards.

Another important method for evaluation of antioxidant activity is RPA method. A compound's reducing capability may indicate its antioxidant potential (Meir et al. 1995). The reduction of Fe_3^{+} 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 $\mu\text{g/mL}$ where the minimum absorbance's were 0.192, 0.183 and 0.175 at 1.953 $\mu\text{g/mL}$ respectively (Table 3). Again, the EC_{50} value for BHT, AA, IHS were 50.84, 23.12, and 279.58 $\mu\text{g/mL}$ respectively. This indicate 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	+
	Salkowski's Test	+
Steroids	Lieberman-Burchard test	+
	Frothing test	+
Saponins	Borntrager's test	-
Anthraquinones	Benedict's test	+
Carbohydrates	Molish's Test	+
	Tannin	+
Flavonoid	Elimination the fatty components	+
	Shinoda's Test	+
	Salkowski Test	-
Tarpinoid	Lieberman-Burchard test	-
Triterpenoid	Ferric chloride test	+
	Gelatin test	+

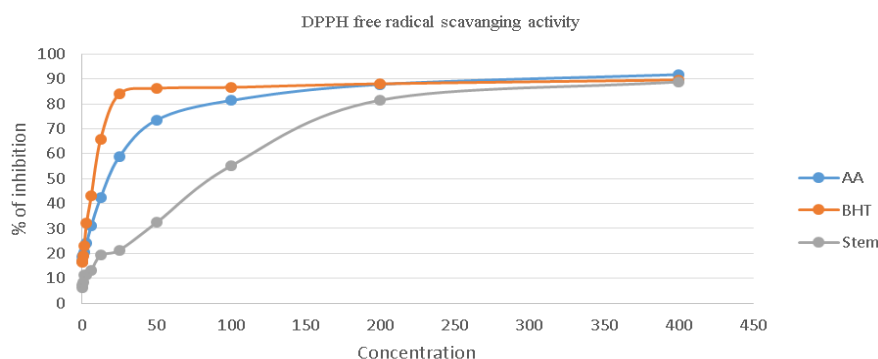
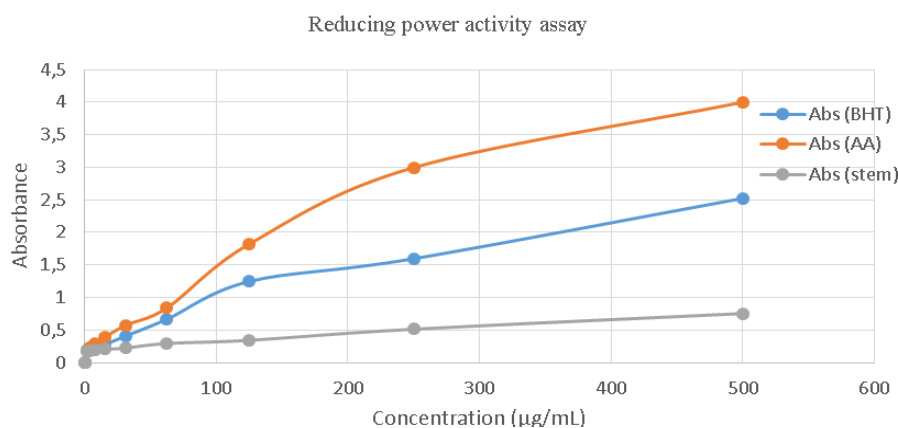
Note: (+) Indicates the presence and (-) indicates the absence of a chemical compound in the sample

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

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

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

Concentration ($\mu\text{g/mL}$)	Avg. absorbance			EC ₅₀ ($\mu\text{g/mL}$)		
	BHT	AA	IHS	BHT	AA	IHS
1.953125	0.192±0.004	0.183±0.024	0.175±0.001	50.84	23.121	279.58
3.90625	0.204±0.026	0.222±0.002	0.185±0.022			
7.8125	0.258±0.034	0.299±0.008	0.192±0.014			
15.625	0.286±0.008	0.393±0.012	0.205±0.001			
31.25	0.41±0.043	0.576±0.004	0.226±0.005			
62.5	0.668±0.052	0.84±0.001	0.295±0.024			
125	1.247±0.038	1.82±0.043	0.344±0.012			
250	1.597±0.004	2.996±0.028	0.514±0.008			
500	2.523±0.006	4.12±0.001	0.755±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 for the treatment of a wide variety of illnesses. At present, the study of phyto-pharmacology has opened up a new field for the discovery of 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 (Uddin et al. 2020). It is believed that around thirty percent of medications are generated from plant compounds (Uddin et al. 2020). Our study has identified the presence of bioactive compounds such as alkaloids, cardiac glycosides, flavonoids, steroids, saponin, tannins, and phenols in *I. hederifolia* stem. These bioactive compounds are responsible for curing tons of diseases. Several studies revealed that phenols and flavonoids are the phytochemical 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 *I. hederifolia* stem. Therefore, we can claim that *I. hederifolia* stem belongs to good antioxidant activity. We hope, researchers will isolate new pharmacological active compound 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. Additionally, in compared to a reference standard, the current investigation also found that IHS had modest antioxidant activity. A high concentration of phenolic compounds, as well as other phytochemicals, might be linked with plant's antioxidant effects. So, extracting the plant's bioactive components might be a viable alternative to synthetic antioxidants.

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