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Original Article

Anti-Inflammatory and Antioxidant Potentials of *Merremia hederacea* (Burm.Fil) Leaves

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Abstract

Background and Aim: This study aimed to identify the existence of phytochemical compounds and evaluate the *in-vitro* antioxidant and anti-inflammatory potentials of the leaves extract of *Merremia hederacea* which is commonly known as ivy woodrose.

Materials and Methods: The antioxidant potency was measured using DPPH radical scavenging method and reducing power capacity followed by calculating phenol and flavonoid contents. To test the anti-inflammatory effect, the protein-denaturation method was applied.

Results: Based on the DPPH scavenging activity, the IC_{50} value of the extract was determined to be 416.977. The amounts of phenolic content and flavonoids as well as the reducing power of this extract were found satisfactory. The extract remarkably hindered the denaturation of protein in the anti-inflammatory activity test with a maximum of 68.86% inhibition at 500 µg/mL concentration.

Conclusion: The results indicate that *M. hederacea* leaves have favorable antioxidant and anti-inflammatory potencies; hence, this plant can be an effective source of new potent drugs.

Keywords: *Merremia hederacea*, Plant extracts, Phytochemicals, Antioxidants, Anti-inflammatory

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Introduction

The World Health Organization evaluates that around eighty percent of the total population utilize natural medications for some parts of essential social insurance. Countless plants are being utilized as prescriptions or as crude materials for different pharmaceutical arrangements (1, 2). A considerable lot of the cutting-edge significant medications are of the plant starting point (3). Several of medications currently used throughout the world such as digitoxin, atropine and opiate antinociceptive medications such as morphine, codeine etc. have been created from plants. Moreover, thousands of plant metabolites are largely effectively utilized in the treatment of different illnesses depended on the use of natural or customary drugs. A couple of significant models are Taxol from Taxusbrevifolia, Vincristine, and Vinblastine from Vincorosea Linn. (Periwinkle plant), and the digitalis glycosides which is cardiotonic in man was derived from foxglove. The goal of the plant-based research is focused on the isolation of new potent and bioactive plant metabolites, such as lead substances (4, 5).

An antioxidant is a chemical that attenuates the

oxidative injury and therefore will suppress or reduce the chance for developing several age-associated free radical tempted disorders. The curiosity in antioxidants derived from plants has inflated recently because the chance of toxicity of artificial antioxidants has been disapproved (6).

Merremia hederacea (Burm.Fil) (Family: Convolvulaceae) is a perennial flowering plant with slender, annual, twining or prostrate stems. This plant is indigenous to tropical Africa, China, India, Bangladesh, tropical Australia, Southern Asia, and Malaysia. It is also found near moist places, along river banks and ponds. *Merremia hederacea*, which is harvested for local use as a medicine (7), is used medicinally for the treatment of acute tonsillitis. The leaves of this plant are used to heal fractures in the arms and feet.

Materials and Methods

Plant parts collection and proper identification

The leaves of *M. hederacea* have been gathered from Fatikchari, Bangladesh in March 2019 at the mature stage. Then, the plant was identified by Professor Shaikh Bakhtiar Uddin, Department of Botany, University of Chittagong, Bangladesh. The plant parts were then dried in shade around 30ºC for one week. Subsequently, the leaves were dried at a medium temperature in a mechanical oven. Then, the plant parts were ground and allowed to pass through a sieve having mesh size 60. Finally, the fine powder was collected.

Extraction Process

About 800 g of the fine powder of leaves of *M. hederacea* was taken in a dry flask (capacity 5 L) and doused in 4 L of methanol for two weeks at ordinary temperature with periodic shaking. Subsequently, the blend was filtered initially with a cotton plug and then with a filter paper. Then, the filtrate was concentrated at 45ºC to remove the solvent. At the end of this process, a solid residue was collected.

Phytochemical Screening

For the detection of each class of compound, there are various phytochemical screening processes. Following the procedures mentioned in the standard guideline(8), initial qualitative phytochemical screening was performed for the detection of alkaloids, carbohydrate, glycosides, tannins, flavonoids, saponins, phenols, and proteins.

Anti-inflammatory Test

The anti-inflammatory effect has been evaluated following the procedure mentioned by HM Arif Ullah(9). Egg was used as the source of albumin and was reconstituted as 5% v/v aqueous solution with iso-saline. Phosphate buffer solution (pH 6.4) was prepared by mixing the required amount of Na_2HPO_4 and NaH_2PO_4. The test solution of variable concentrations (100-500 µg/mL) was prepared in distilled water. The standard aspirin solution was prepared in the respective concentration in distilled water. Each test tube contained egg albumin (0.2 mL), phosphate buffer solution (2.8 mL) and extract solution or the standard solution of varying concentrations. The same volume of distilled water has been used as the control solution. Finally the mixtures were kept at a temperature around 37°C in an incubator for a period of 15 min, and then the temperature was raised to 70°C for 5 min. The absorbance was recorded at 660 nm after cooling against the blank (10). The percentage inhibition of protein denaturation was determined using the following formula:

% inhibition = [(absorbance of the control solution - absorbance of the extract solution) ÷ absorbance of the control solution] ×100

DPPH Free Radical Scavenging

The ability to scavenge the free radicals of the extract was determined using DPPH. The absorbance was recorded by the spectrophotometer at 517 nm(11). For the preparation of 0.004% (w/v) DPPH solution, 4 mg of measured DPPH was liquefied in 100 mL of 95% methanol in a dark room. The standard solution (ascorbic acid solution) was prepared by dissolving 2mg of the ascorbic acid in 2.5mL distilled water (concentration 800 mg/mL). From this solution, different concentrated solutions (31.25mg/mL, 62.5mg/mL, 125mg/mL, 250mg/mL and 500mg/mL) were prepared. To prepare the test solution, 5mg extract of *M. hederacea* leaves was dissolved into 10 mL of methanol (concentration 500µg/mL). Then, sequential dilution was carried out to prepare desired concentrated solutions. Likewise, 3mL of DPPH solution was served as the negative control and methanol was served as the blank.

In the test tube, 2 mL of different concentrations of the extract solution or standard was taken in the test tubes

containing 3 mL of DPPH solution. After that, the test tubes were placed into an incubator for 30 minutes at the room temperature to finish the reaction. The absorbance was determined at 517 nm by a spectrophotometer. Except for the extract solution or the standard solution, a blank solution had all other reagents. By the following equation, the % inhibition was quantified using the following formula:

% I = [(Ao – A1)/Ao] X 100

Where, Ao = absorbance of the control, and A1 = absorbance of the extract/standard. Then, the IC_{50} was measured.

Reducing Power Capacity

Using the process of Oyaizu (1986), the reducing power of *M. hederacea* was estimated following the process mentioned by Ferreira (12). To prepare a 1% solution of potassium ferricyanide in a volumetric flask, 1 gm of $[K_3Fe(CN)_6]$ was taken and then the volume was adjusted using distilled water up to 100mL. To prepare a 10% solution of the trichloroacetic acid solution, trichloroacetic acid (10 g) was taken and then the volume was adjusted using distilled water up to 100mL. In the case of preparing a 0.1% solution of ferric chloride, ferric chloride (0.1 g) was mixed in 100mL distilled water. To prepare 0.2M phosphate buffer, K_2HPO_4 (3.48 g) and KH_2PO_4 (2.72 g) were mixed with distilled water (100mL). Subsequently, the pH was adjusted. The standard ascorbic acid solution (1mg/mL concentration) was composed of 10mg of the ascorbic acid in 10mL distilled water. From this solution, several concentrated solutions (31.25- 500µg/mL) were prepared. The extract solution was prepared by dissolving 5mg *M. hederacea* leaf extract into 10 mL of methanol (concentration 500µg/mL). Then, sequential dilution was carried out to prepare desired concentrated solutions.

To perform the experiment, 1.0 mL of the prepared extract solution or standard was taken at various concentrations in the test tubes. Then, 0.2M phosphate buffer (2.5 mL) and potassium ferricyanide (2.5 mL) solution were added into the test tube. For completing the reaction, the test tubes were kept at 50ºC for 20 minutes. Subsequently, 2.5 mL of trichloroacetic acid solution was incorporated. After that, the mixtures were centrifuged for 10 minutes at 3000 rpm. From the upper part of the test tubes, 2.5 mL supernatant was withdrawn and 2.5mL distilled water was added. To dilute the mixture, 0.5 mL 0.1% $FeCl_3$ solution was mixed. The absorbance of each solution mixture was recorded at 700 nm by the spectrophotometer against the blank.

Measurement of Total Phenol Content (TPC)

The TPC of the extract of *M. hederacea* was measured employing the procedure explained by Singleton and Rossi that involved oxidizing agent Folin-Ciocalteau reagent (FCR) and standard Gallic acid(13, 14). For the preparation of FCR reagent, 2 mL of FCR reagent was added into 18 mL distilled water in a beaker for 10 times dilution. To prepare a 7.5% Na_2CO_3 solution, 7.5 gm of Na_2CO_3 was mixed with distilled water and finally the volume was adjusted to 100mL. Standard Gallic acid solution (1000 µg/mL concentration) was constituted by mixing 1mg of the Gallic acid and 1mL of distilled water. From this stock solution, several concentrated solutions (62.5-500µg/mL) were prepared. The blank solution was prepared using 5 mL of FCR, 1 mL of methanol and 4 mL of Na_2CO_3 solution. For preparing the sample solution of 500µg/mL concentration, 5mg of the methanolic extract of *M. hederacea* leaves was liquefied in 10 mL of methanol.

For the experiment, 0.5 mL of the extract solution or standard of the various concentrations was taken in the test tube. 10 times diluted 2.5 mL of FCR solution was mixed into the test tube. Then, 2.5 mL of 7.5% Na_2CO_3 solution was poured into the test tube. To complete, the reaction, all the test tubes were kept at 25ºC for 20 minutes. Using spectrophotometer, the absorbance was determined at 760 nm against the blank. The TPC in the extract was measured by the formula: A = (c x V)/m

Where, A= total phenolic content, mg/g extract, in Gallic acid equivalent; c = the Gallic acid concentration (from the standardization curve), V = extract volume in mL; m = pure plant extracts weight, g per mL.

Measurement of Total Flavonoid

The measurement of the flavonoid content of *M. hederacea* leaves was done by $AlCl_3$ which was known as the colorimetric method. Here, quercetin was used as the standard. (15, 16). 10% $AlCl_3$ solution was prepared mixing 10 g $AlCl_3$ into 100mL distilled water. 1M CH_3COOK solution was prepared by mixing 9.8gm potassium acetate into 100mL distilled water. Standard quercetin solution (1000 µg/mL concentration) was

prepared by adding 1mg of the quercetin in 1mL distilled water. From this solution, several concentrated solutions (12.5-100µg/mL) were prepared. The blank solution contained each of the reagents, except the extract or standard which is replaced with 0.25 mL of methanol. The extract solution was prepared in methanol (concentration 500 µg/mL).

For the experiment, the extract solution (500 µg/mL) or standard (1.0 mL) of different specific concentrations was taken in the test tube. Then, 3 mL of methanol was incorporated. Subsequently, 10% $AlCl_3$ solution (200µl) was mixed into the test tube. After that, 200µl of potassium acetate (1M) and 5.6mL distilled water were incorporated. To complete the reaction, the test tube was kept into the incubator at room temperature. The incubation completed after 30 minutes. The absorbance was determined at 420 nm against the blank using the spectrophotometer. The total amount of the content of flavonoid compounds was measured by the formula C= (c x V)/m

Where, C = total flavonoid content in mg per g of the extract, in quercetin equivalent (QE); c = the quercetin concentration found from the standardization curve, mg/mL; V = extract volume in mL; m = pure plant extracts weight, g per mL.

Statistical Analysis

Data has been represented as mean±SEM. The one-way ANOVA followed by Dunnet's text was applied to interpret the remarkable distinctions between the sample or standard and the control group using GraphPad Prism, version 6. P values found were considered to be statistically significant ($p<0.05$).

Results and Discussion

To ensure the existence or nonexistence of the secondary metabolites of the plant, the qualitative phytochemical screening was implemented. The results showed the existence of alkaloids, carbohydrates, glycosides, flavonoids, tannins, saponins and phenolic compounds in the plant extract (Table 1).

Alkaloids are various classes of secondary metabolites found in plants. They have been used for therapeutic purposes for centuries. They have potential antioxidant properties which can suppress chronic degenerative diseases (17). Furthermore, the majority of the alkaloids are able to exert significant anti-inflammatory activities (18). Glycosides are also a group of plants' secondary metabolites and are very effective for treating heart-related diseases (19). Phenolic compounds are considered to be very useful plant metabolites for men. They have a wide range of beneficial properties, including antioxidant, anti-inflammatory, anti-

Table 1: Phytochemical Screening of methanolic extract of *M. hederacea*.

Sl. no.	Test Name	Observation
1	Alkaloid test	
	i. Mayer Test	++
	ii. Wagner Test	_
2	Carbohydrate test	
	i. Molish Test	+
	ii. Benedicts Test	+
	iii. Fehling A&B	_
3	Glycosides Test	+
4	Flavonoids Test	++
5	Tannins Test	+
6	Saponins Test	+
7	Phenols Test	++
8	Protein Test	-
9	Terpin Test	_
10	Cholesterol Test	_

Table 2: Anti-inflammatory activity of methanolic extracts of *M. hederacea*.

Test sample		Standard drug	
Conc. (µg/ml)	**Mean% inhibition ± SD**	**Conc. (µg/ml)**	**Mean% inhibition ± SD**
500	68.86± 2.74	500	80.64 ± 0.91
250	65.66 ± 3.02	250	74.19 ± 1.43
125	61.47 ± 1.77	125	65.51 ± 1.43
62.5	42.11 ± 2.22	62.5	43.11 ± 1.03

Table 3: DPPH radical scavenging assay for the methanolic extract of *M. hederacea* leaves and standard ascorbic acid.

Sample		Standard	
Concentration(µg/ml)	**% SCV**	**Concentration(µg/ml)**	**% SCV**
31.25	8.208±1.103	31.25	32.032±1.15
62.5	21.522±1.77	62.5	46.246±2.20
125	38.539±1.15	125	93.694±2.50
250	44.545±1.09	250	96.496±0.79
500	50.751±1.97	500	97.497±1.12

Table 4: Reducing power capacity of the standard ascorbic acid and the methanolic extract of *M. hederacea* leaves.

Serial no.	Concentration (µg/ml))	Absorbance of ascorbic acid	Absorbance of sample
1	31.25	0.874±0.011	0.089±0.013
2	62.50	1.472±0.021	0.195±0.087
3	125	2.1±0.032	0.432±0.045
4	250	2.364±0.021	0.645±0.024
5	500	2.587±0.056	0.955±0.028

carcinogenic, anti-hypertensive, anti-allergic, anti-arthritic, and anti-bacterial effects. Moreover, they are therapeutically used for the treatment of cancer, diabetic, obesity and infectious diseases (20). It has been reported that these sorts of phenolic compounds are capable of reducing the production and effects of pro-inflammatory substances inside the body which result in an anti-inflammatory action (21). Flavonoids are polyphenol types of compounds having beneficial effects on human health. They are also well known for their antioxidant, anti-inflammatory as well as anticancer properties (22). Tannins are a sub-class of phenolic compounds of high molecular weight. They are used as herbal medicines for their wound healing effect. They are effective antidotes for metallic, alkaloidal and glycoside poisonings. Studies have also revealed their antioxidant, anti-microbial and antiradical properties (23). Saponins exhibit anti-microbial, anti-fungal, anti-allergenic, anti-diarrheal and anti-cancer activities (24).

The findings of the anti-inflammatory test have been represented in Table-2. The methanol extract of *M. hederacea* showed 23.70, 23.70, 53.04, 64.43, and 77.15% of protein denaturation for doses of 100, 200, 300, 400, and 500 µg/mL respectively; whereas for the standard, the values were 50.56, 69.58, 77.18, 80.25 and 88.06% respectively. The methanolic extract showed favorable anti-inflammatory effects with a linear dose-response relationship. The result was considered significant with $p<0.05$.

The results for the radical scavenging capacity of *M. hederacea* leaves extract have been indicated in Table 3. A dose-reliant radical scavenging effect in the DPPH assay was showed by the extract. The IC_{50} value of the sample was 416.977 µg/mL that is statistically substantial ($p<0.05$) and assimilated to the ascorbic acid

(IC_{50} 193.548 µg/mL). Hence, by comparing with the standard ascorbic acid, it can be claimed that the plant parts possess anti-radical activities.

Reducing power results of the standard ascorbic acid as well as extract of *M. hederacea* leaves have been shown in Table 4. The reducing power for the extract was improved by increasing sample concentration. Reducing capacity is normally related to the occurrence of reducing agents capable of exerting antioxidant action through infringement of the free radical by releasing an H-atom (25, 26).

The phenolic compounds are free radicals terminators. The phenolic content of the test extract was quantified by the standard calibration curve found from Gallic acid. In this experiment, the total phenolic content of the extract was found to be 40.19±0.599mg gallic acid/gm of the extract. As previously mentioned, phenolic compounds are the most important plant metabolites due to their scavenging ability. Hence, phenolic components might be highly influential on the antioxidant effect in the present study

In this investigation, the flavonoid content of the *M. hederacea* leaves was quantified as quercetin equivalent using the standard calibration curve. The total amount of the flavonoid content of *M. hederacea* leaves was 46.67 ± 0.23 mg quercetin equivalent/gm of the extract.

This preliminary phyto-pharmacological phytochemical analysis shows that the plant *M. headracea* has considerable antioxidant and significant anti-inflammatory capacities. It can be claimed that these effects are directly correlated with the existence of important phytochemicals such as phenolic compounds, flavonoids, tannins, and saponins in the plant materials.

Conclusion

From the findings of these investigations, it can be concluded that antioxidant as well as anti-inflammatory effects of the extract of *M. hederacea* leaves might be the direct result of the existence of various chemical constituents in this plant. In these studies, phytochemical screening was carried out for justifying various phytochemicals present in the plant extract. Depending on the experimental evaluation, it can be claimed that this plant can be a potential source of new potent drugs and herbal medicine. Hence, further inquiry is required to determine the active compounds which might be responsible for such effects.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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